IE 921782

PATENTS ACT, 1964

COMPLETE SPECIFICATION

TETRAVALENT BISPECIFIC RECEPTORS, THE PREPARATION AND USE THEREOF

APPLICATION No. 921782

SPECIFICATION FILED 1/7/92

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Description

Tetravalent bispecific receptors, the preparation and use thereof

The invention relates to tetravalent bispecific receptors which are prepared by genetic manipulation by fusion of the DNA which codes for the heavy chain of an F(ab')2 fragment of an antibody (I) with (a) DNA which codes for the heavy chain of an F(ab')2 molecule of a second antibody (II), in which the CH1 domain is replaced by a CH3 domain (formula I), or with (b) the DNA which codes for a single chain FV fragment of an antibody (II) (formula II), by means of suitable linkers. The expression of these fusion genes in mammalian cells together with the genes for the corresponding light chains, which in the case of the construct (a) are composed on the one hand of a VL exon of specificity I and a CK exon, and on the other hand of a VL exon of specificity II and a CH3 exon, and in the case of the construct (b) only of a VL exon of specificity I and a CK exon, yields tetravalent bispecific receptors. In this case the CH1 domains are connected to the VH2 domains via 1 to 10 hinge regions (H) and a suitable peptide linker L. The antibody specificities described in European Patent Application EP-A2-0404 097 are preferably employed. They are, inter alia, on the one hand specificities which are directed against an epitope, which is located on the cell membrane or in the interstitium of a tumor-associated antigen. On the other hand, these are specificities which are directed against high or low molecular weight ligands which in turn bind an agent which is active against tumors, or bind this active agent directly.

EP-A2-0404 097 describes bispecific and oligospecific, mono- and oligovalent receptors which are prepared by genetic manipulation by fusion of DNA coding for F(ab) fragments of antibodies of two or more different specificities by means of suitable linkers. In this case, one specificity is preferably directed either against an epitope, which is located on the cell membrane or in the interstitium, of a tumor-associated antigen (TAA) or against an epitope in the tumor endothelium (TE), while the other specificities relate to high or low molecular weight ligands and react, for example, with the complexons ethylenediaminetetraacetate and diethylenetriaminepentaacetate in yttrium-90-complexed form (EDTA-90Y and DTPA-90Y respectively). In a particularly preferred embodiment, the binding to the complexons takes place on the complexon receptor arm via fos-jun interaction (or else avidin-biotin interaction). Other preferred specificities have catalytic properties.

Bispecific antibodies have hitherto been prepared by the following methods

- chemical coupling of antibodies of different specificity via heterobifunctional linkers (H. Paulus, Behring Inst. Mitt. 78, (1985), 118-132)

- fusion of hybrids which are already available and which secrete different monoclonal antibodies (MAbs), and isolation of the bispecific monovalent portion (U.S. Staerz and M.J. Bevan, Proc. Natl. Acad. Sci. USA 83, (1986) 1453-1457)

- transfection of the light and heavy chain genes of two different MAbs (4 genes) into murine myeloma cells or other eukaryotic expression systems and isolation of the bispecific monovalent portion (U. Zimmermann, Rev. Physio. Biochem. Pharmacol. 105

(1986), 176-260; J. van Dijk et al., Int. J. Cancer 43, (1989), 944-349).

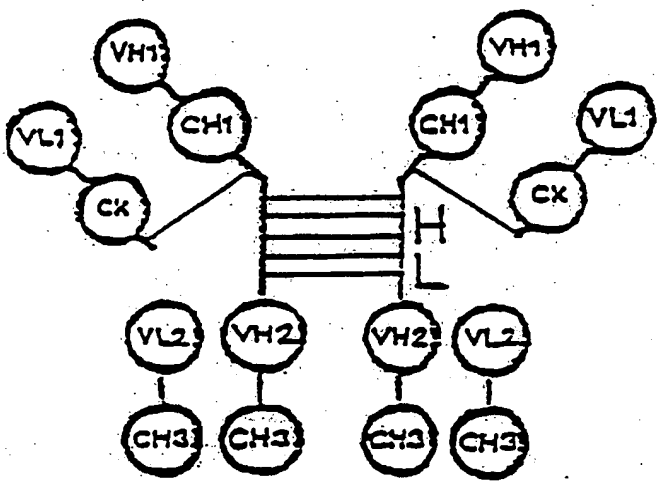
Such bispecific antibodies are employed for the therapy and diagnosis of malignant tumors. The principle of the method comprises in the first step a saturation of the epitopes on the target cells which are recognized by one of the two specificities on by multiple high dose injections of the bispecific macromolecule. In the second step, which comprises interruption of the treatment for several days, autoelimination of the non-specifically adsorbed bispecific antibodies from the non-target tissues takes place.

This autoelimination can be speeded up by injection of an anti-idiotype antibody which is coupled to sugar residues, preferably galactose, and which is directed against the anti-tumor arm of the bispecific receptor.

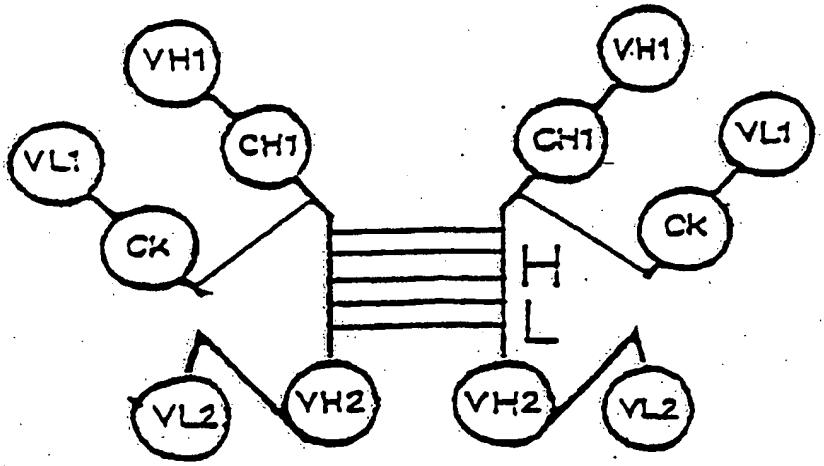
The third step in the method comprises i.v. injection of a radiolabeled, hydrophilic, low molecular weight ligand which does not accumulate in cells, has a short residence time in the body, has high complexation constants for beta- and gamma-emitters such as 90Y, 186Re, 188Re, 189Re, 99mTc or 111In and binds with high affinity to the second specificity of the bispecific antibody. This step achieves accumulation of the radioactive ligand, together with a longer residence on the target tissue, which results in the selective destruction of the target tissue or makes diagnosis possible, for example of metastases.

We have found that tetravalent bispecific receptors of the formula I or formula II can be prepared particularly well by genetic manipulation because they are expressed considerably more efficiently than bispecific antibodies generated by other methods. An additional factor is that

the avidity of the original antibodies for their corresponding antigens is retained in the constructs according to the invention. Preferred constructs have 1 to 5, very preferably 1, hinge region between CH1 and VH2, because such constructs are expressed particularly efficiently in BHK cells. Preferably employed as linkers are the sequences corresponding to the peptide sequence (Gly-Gly-Gly-Gly-Ser)x with x = 3 to 5 or GEAAPAAAPAAAAAGG. Otherwise, the antibody V gene fragments or specificities described or preferred in the abovementioned EP-A2-0404 097 are also preferably employed in this case.



formula I



formula II

H = hinge
L = linker
—— = peptide linkage
___ = dilsulfide bridge

The construction of a fusion gene which codes for a tetravalent bispecific receptor molecule is described by way of example hereinafter. Unless otherwise noted, the techniques used for this are taken from the book "Molecular Cloning: A Laboratory Manual" (T. Maniatis et al., 1989). Further details are described in EP-A2-0404 097, to which particular reference is made here.

The present invention accordingly relates to bispecific tetravalent receptors of the formula I or formula II, to processes for the preparation thereof and to the use thereof. It is preferable for one specificity to be directed against animal or human tumor-specific antigens and the other specificity to have catalytic or enzymatic activity or be directed against a complexing agent. In another preferred embodiment, one specificity derives from the monoclonal antibodies with the variable regions shown in Tab. 2, 3, 4 or 5 in EP-A2-0404 097.

Example 1: A derivative of an M13 phage (VH PCR) which contains the 5′ part of a heavy chain gene composed of promoter region, signal exon, intron 1, VH exon and intron 2 (R. Orlandi et al., Proc. Natl. Acad. Sci. USA, Vol. 86, 3833-3837, 1988) was cleaved with the restriction endonucleases HindIII and BamHI, and the insert was isolated and cloned into a HindIII/BamHI-cleaved KS+ phasmid (pBluescript KSR+, Stratagene LaJolla, CA, USA). The phasmid clone (A) which contains the insert cut out of the VH PCR was identified by restriction analysis and nucleic acid sequence analysis (Fig. 1).

Example 2:
The phasmid clone (A) was cleaved with XbaI and HindII, the XbaI cleavage site was made blunt ended using polymerase and dNTPs, and the DNA fragment with the V gene exon was isolated. The isolated DNA fragment was then cloned into a KS+ phasmid from which the polylinker and the regions adjacent to the polylinker had been deleted by PvuII digestion. The clone (B) which contains the VH insert and the regions of the KS+ polylinker located between the XbaI and BamHI or HindII and HindIII cleavage sites was identified (Fig. 2). The phasmid B was used to clone the VH genes of the antibodies I and II (VH1 and VH2) after amplification from the cDNA of the hybrid cells. Amplification of the VH genes was carried out by the method described by R. Orlandi et al. (1989, loc. cit.). The phasmid clone B with the VH1 gene is called B1, and the phasmid clone B with the VH2 gene is called B2 (R.M. Hudziak et al., Cell., Vol. 31, 137-146, 1982; F. Lee et al., Nature, Vol. 294, 228, 1981).

Example 3:
A pUC 19 plasmid which contains the CH1 exon and the first hinge exon of a human IgG3 gene (EP-A2-0404 097; Fig. 3 ibidem: IgG3 (F(ab′)2 1H)), was cleaved with the restriction endonuclease HindIII, and the

cleavage sites were made blunt ended with Klenow DNA polymerase. This was followed by partial cleavage with PstI, and the DNA fragment with the CH1 exon and the H1 exon was isolated and cloned into a pUC 18 plasmid cleaved with PstI and HindII. The clone (C) which harbors a BamHI cleavage site 5′ of the insert and a HindIII cleavage site 3′ of the insert was isolated (Fig. 3).

Example 4:
The plasmid clone C was cleaved with HindIII, and the cleavage sites were filled in with Klenow DNA polymerase. Then the insert with the CH1 and H1 exons was cut out with BamHI, isolated and cloned into a KS+ phasmid (pBluescriptR IIKS+, Stratagene, LaJolla, CA, USA). The KS+ phasmid had been cleaved with XbaI, the XbaI cleavage sites made blunt ended in with Klenow DNA polymerase and subsequently cut with BamHI. The phasmid clone (D) which contains the insert with the CH1 and hinge 1 exons in an orientation in which a BamHI and a HindIII cleavage site are located on the 5′ side of the insert was isolated (Fig. 4).

Example 5:
The phasmid clone (B1) was cleaved with HindIII and BamHI, and the insert with the VH gene of antibody I was isolated and cloned into the vector (D) which had likewise been cleaved with HindIII and BamHI. The clone (E) which contains the VH1 gene, the CH1 and the hinge 1 exon was isolated (Fig. 5).

Example 6:
A DNA fragment (F) which contains a VH gene extended by a linker and harbors at its 5′ end a cleaved PvuII cleavage site and at its 3′ end a BamHI cleavage site (Fig. 6) was amplified from the phasmid vector B2 by the polymerase chain reaction (PCR) using the oligonucleotides VHOligoI and VHOligoII (Tab. 1). This fragment was

cleaved with BamHI and cloned into a BamHI/PvuII cleaved KS+ vector in which one of the two internal PvuII cleavage sites had previously been destroyed by Asp718/-PvuII cleavage, filling-in of the cleavage sites and religation.

Tab. 1:

VH OligoI: _____

PvuII

5' CT.GCC.GCC.CCC.GCA.GCC.GCA.

GCC.GCA.GGC.GGC.CAG.GTC.CAA.CTG.CAG.GAG.

3'

AGC.GGT.CCA.GG

VH OligoII: _____

BamHI

5' 3'

CGG.GGA.TCC.TAT.AAA.TCT.CTG.GC

The phasmid clone (F) which contains the amplified fragment was isolated (Fig. 6).

Example 7:

The oligonucleotides OligoIII and IV (Tab. 2) were annealed together, and the resulting DNA fragment was ligated into the phasmid clone (F) cleaved with PvuII (Fig. 7). The phasmid clone (G) which contains a fusion exon composed of a hinge exon, an oligonucleotide linker and the VH2 gene was isolated.

Tab. 2:

Oligo III:

5′

Hind III Sph I

GCG.GAA.GCT.TCG.GGC.ATG.CTA.ATC.TTC.TCT.CTT.GCA.GAG.

CCC.AAA.TCT.TGT.GAC.ACA.CCT.CCC.CCG.TGC.CCA.AGG.TGC.

CCA.GGA.CAG

3′

Oligo IV:

5′

CTG.TCC.TGG.GCA.CCT.TGG.GCA.CGG.GGG.AGG.TGT.GTC.ACA.

AGA.TTT.GGG.CTC.TGC.AAG.AGA.GAA.GAT.TAG.CAT.GCC.CGA.

AGC.TTC.CGC Sph I

3′

Hind III

Example 8:

The CH3 exon and 3′ NT region of the IgG3 gene were amplified out of the plasmid clone 54.1.24, which contains a human IgG3 C gene (EP-A2-0404 097, Fig. 2), with the oligonucleotides V and VI (Tab. 3) and cloned into the BamHI and EcoRI cleavage sites of a pUC 19 plasmid (Fig. 8). The plasmid clone (H) which contains the CH3 exon of the IgG3 gene was isolated.

Tab. 3:

Oligo V:

5′ BamHI

3′

CC.TCT.GCC.CTG.GGA.TCC.ACC.GCT.GTG.CC

Oligo VI:

5′ EcoRI

3′

AAC.CAT.CAC.GAA.TTC.ACA.GGG.GCC

Example 9:
The plasmid clone (H) was cleaved with BamHI and EcoRI, and the DNA fragment which harbors the CH3 exon was cloned into the phasmid clone (G) cleaved with BamHI and EcoRI (Fig. 9). The phasmid clone (I) which contains the hinge/linker/VH2 fusion exon and the CH3 exon was isolated.

Example 10:
The phasmid clones (E) and (I) were cleaved with HindIII and SphI. The insert of the clone (E) was cloned into the HindIII and SphI cleavage sites of the phasmid clone (I). The phasmid clone (K) containing an Ig heavy chain fusion gene which is composed of signal exon, VH1 exon, CH1 exon, hinge 1 exon, hinge/linker/VH2 MAbII fusion exon and CH3 exon was isolated (Fig. 10).

Example 11:
The HindIII-EcoRI fragment with the fusion gene was cut out of the phasmid clone (K) and cloned into a pAB Stop expression vector (Fig. 19, pAB Stop is a derivative of the pAB 3 vector (G. Zettlmeißl et al., BIM, 82, 26-34,

1988) in which the AT III gene has been replaced by a polylinker) whose BamHI fragment had been replaced by an EcoRI linker. The clone (L) which contains the fusion gene was isolated (Fig. 11). The clone (L) was expanded and used for transfections in mammalian cells.

Example 12:
Construction of light chain gene:
The phasmid clone (B) was cleaved with HindIII and BamHI, and the VH insert was replaced by the VK insert isolated from the vector VKPCR (R. Orlandi et al., 1989, loc. cit.). The phasmid clone (M) which harbors a signal exon and a VK exon was isolated (Fig. 12). The phasmid clone was used to clone the amplified VK genes of MAbs I and II. The vector M with the VK1 gene was called M1 and the vector M with the VK2 gene was called M2.

Example 13:
The human CK gene (Heiter et al., J. of Biol. Chem., 257: 1516-1522, 1982) was isolated as EcoRI fragment and cloned into the SmaI cleavage site of a pUC 19 plasmid. The clone (N) which contains the human CK gene was isolated (Fig. 13).

Example 14:
The clone (N) was cleaved with EcoRI and HindIII, and the CK insert was isolated and cloned into an EcoRI/-HindIII-cleaved KS+ vector. The phasmid clone (O) which contains the CK insert was isolated (Fig. 14).

Example 15:
The clone (O) was cleaved with BamHI, and the CK insert was isolated and cloned into a BamHI-cleaved pAB Stop vector. The clone (P) which contains the CK insert in an orientation in which the 5′ end of the CK gene is in the vicinity of the HindIII cleavage site of the pAB Stop vector was isolated (Fig. 15).

Example 16: The clone (P) was partially cleaved with BamHI, and the cleavage sites were made blunt ended in with Klenow DNA polymerase and religated. The clone (Q) in which the BamHI cleavage site 3' of the CK gene is destroyed was identified (Fig. 16).

Example 17:
The phasmid clone (M1) with the VK1 gene was cleaved with HindIII and BamHI. The insert with the VK gene was isolated and ligated into the HindIII and BamHI cleavage site of the expression vector (Q). The clone (R) which contains an intact kappa light chain gene with the specificity of the antibody I was identified (Fig. 17).

Example 18:
The plasmid clone (H) with the CH3 exon of the human IgG3 gene was cleaved with EcoRI and HindIII, and the CH3 insert was isolated and cloned into an EcoRI/-HindIII-cleaved KS+ vector. The phasmid clone (S) which contains the CH3 insert was isolated (Fig. 18).

Example 19:
The clone (S) was cleaved with BamHI, and the CH3 insert was isolated and cloned into a BamHI-cleaved pAB Stop vector (Fig. 19). The clone (T) which contains the CH3 insert in an orientation in which the 5' end of the CH3 exon is in the vicinity of the HindIII cleavage site of the pAB Stop vector was isolated (Fig. 20).

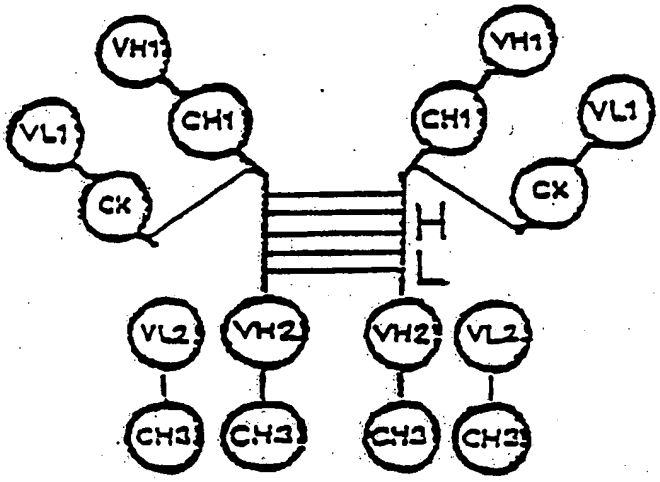
Example 20:
The clone (T) was partially cleaved with BamHI, and the cleavage site was made blunt ended in with Klenow DNA polymerase and religated. The clone (U) in which the BamHI cleavage site 3' of the CH3 gene is destroyed was identified (Fig. 21).

Example 21:
The phasmid clone (M2) with the VK2 gene was cleaved with HindIII and BamHI. The insert with the VK gene was isolated and ligated into the HindIII and BamHI cleavage sites of the expression vector (U). The clone (V) which contains an intact light chain gene with the specificity of the antibody II and a CH3 exon as constant region was identified (Fig. 22).

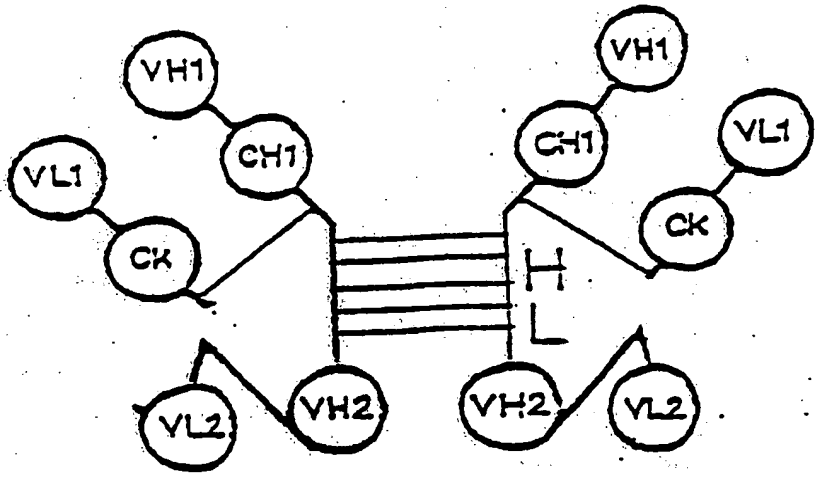
The expression plasmids L, R and V were cotransfected (30) together with suitable phasmids harboring selection markers, such as, for example, pRMH140 (Fig. 23) (R.M. Hudziak et al., 1982, loc. cit.) or pSV2dhfr (Fig. 24) (F. Lee et al., 1981, loc. cit.) into mammalian cells, transfectoma clones were selected by selection pressure, and those transfectoma clones which secrete bivalent tetraspecific receptor molecules were identified by assaying the supernatants with suitable assays.

Patent claims: HOE 91/B 019-Ma 890

1. A bispecific tetravalent receptor of the formula I



or formula II



wherein only the VL and VH pairings which belong together are formed and the CH1 domains are linked to the VH2 domains via 1 to 10 hinge regions H and a suitable peptide linker L, where the disulfide bridges (thin lines) bring about the dimerization of the bivalent halves of the molecule in the region of the hinge regions.

2. A receptor as claimed in claim 1, wherein one specificity is directed against animal or human tumor-associated antigens.

3. A receptor as claimed in claim 1, wherein one specificity has catalytic or enzymatic activity.

4. A receptor as claimed in claim 1, wherein one specificity is directed against animal or human tumor-associated antigens and the other specificity has catalytic or enzymatic activity.

5. A receptor as claimed in claim 1, wherein one specificity is directed against animal or human tumor-associated antigens and another specificity is directed against a complexon.

6. A receptor as claimed in claim 1, 2, 4 or 5, wherein one specificity derives from the monoclonal antibodies with the variable regions shown in Tab. 2, 3, 4 or 5 in EP-A2-0 404 097.

7. A receptor as claimed in claim 1 to claim 6 as pharmaceutical.

8. A process for the preparation of receptors as claimed in claim 1, 2, 3, 4, 5 or 6, which comprises the DNA fragments coding for the heavy chain antibody portions

being connected by linkers and expressed in an expression system together with the genes for the light chains.

9. A bispecific tetravalent receptor of the formula I in which the CH3 domains are replaced by human CH1 domains.

10. A bispecific tetravalent receptor as claimed in claim 1, substantially as hereinbefore described and exemplified.

11. A process for the preparation of a bispecific tetravalent receptor as claimed in claim 1, substantially as hereinbefore described and exemplified.

12. A bispecific tetravalent receptor as claimed in claim 1, whenever prepared by a process claimed in a preceding claim.

Dated this the 2nd day of June, 1992

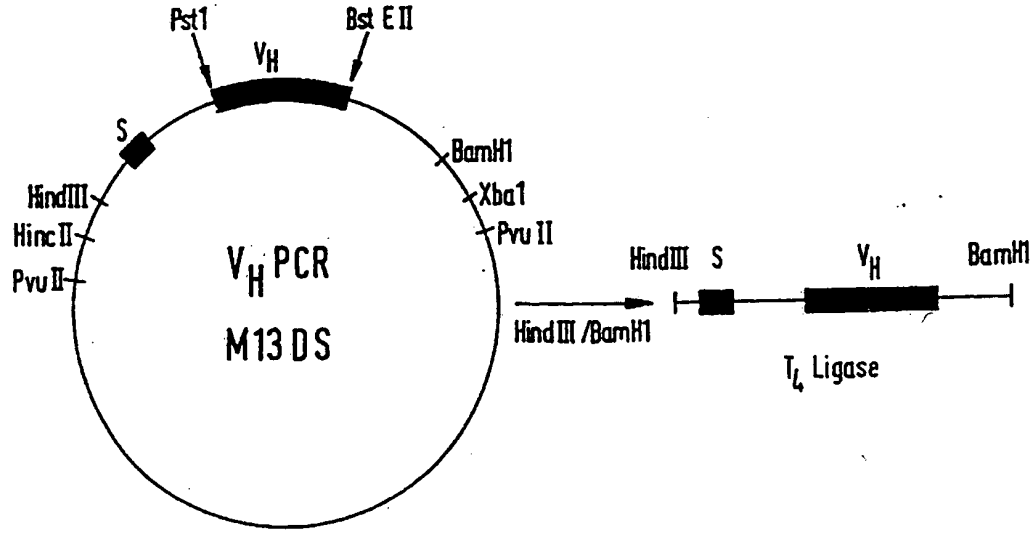
F. R. KELLY & CO.

BY: _____ EXECUTIVE

27 Clyde Road, Ballsbridge, Dublin 4
AGENTS FOR THE APPLICANTS

BEHRINGWERKE AKTIENGESELLSCHAFT 23 Sheets TRUE COPY
Sheet 1

Fig.1



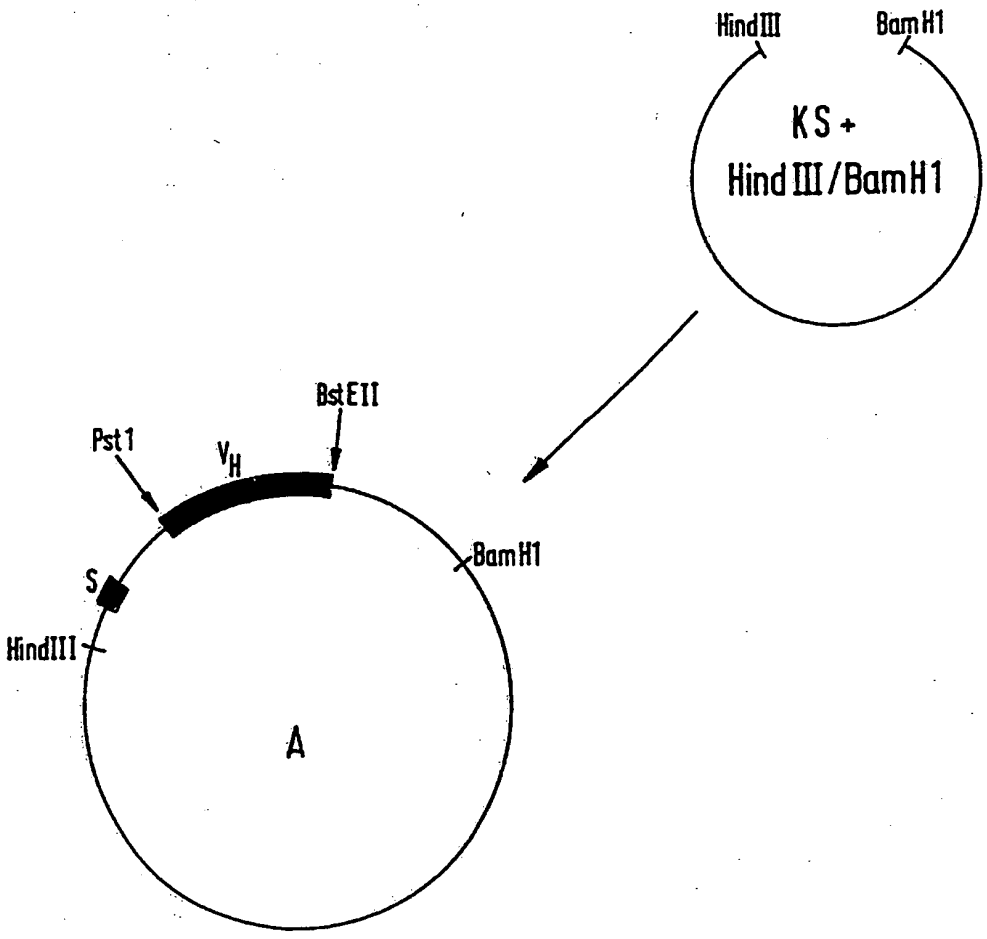


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Fig.2

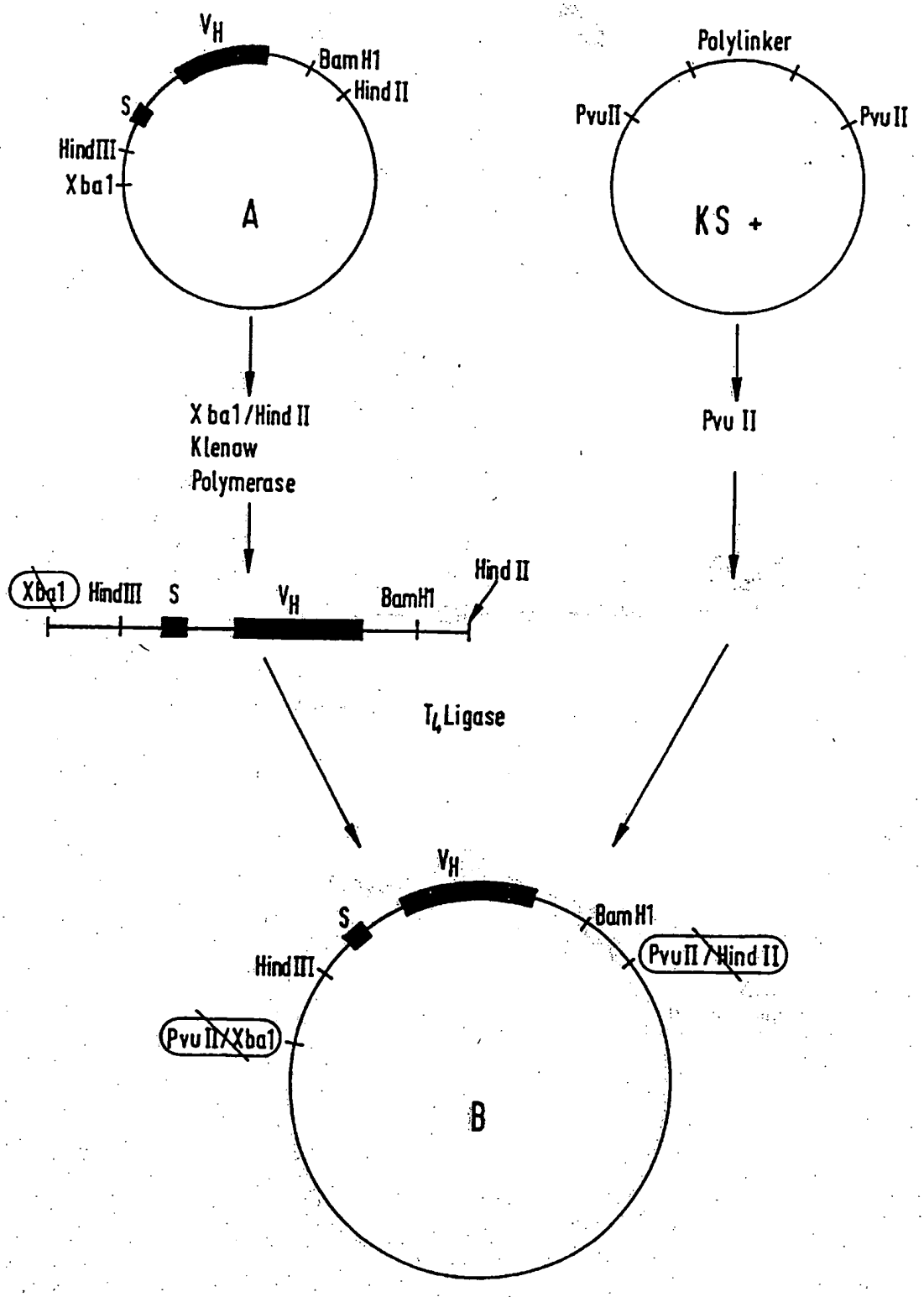
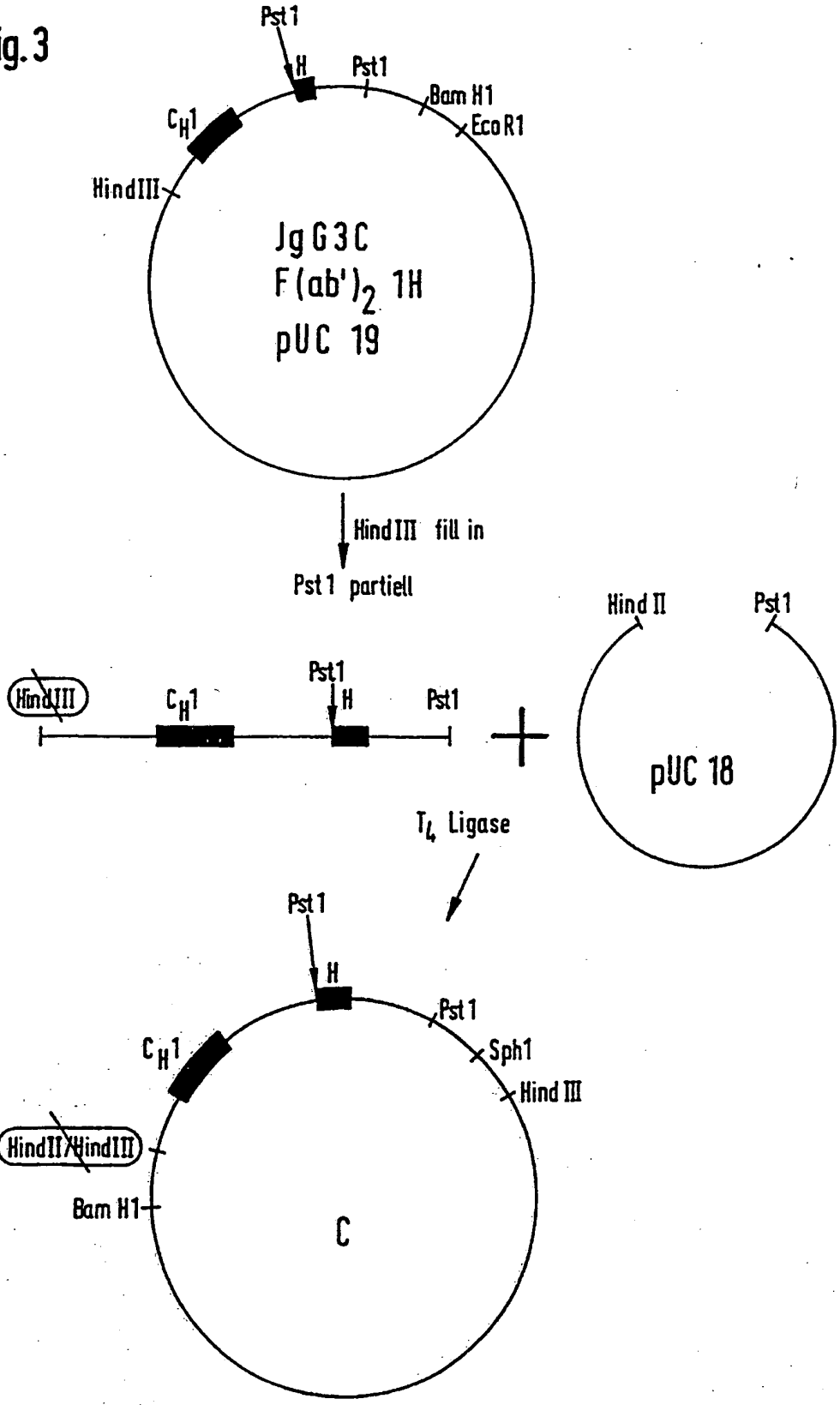

V_H
BamH1
Hind II
S
HindIII
Xba1
A
Polylinker
PvuII
PvuII
KS +
Xba1/Hind II
Klenow
Polymerase
Pvu II
Xba1
HindIII
S
V_H
BamH1
Hind II
T_4 Ligase
V_H
S
BamH1
HindIII
PvuII/Hind II
PvuII/Xba1
B

Fig. 3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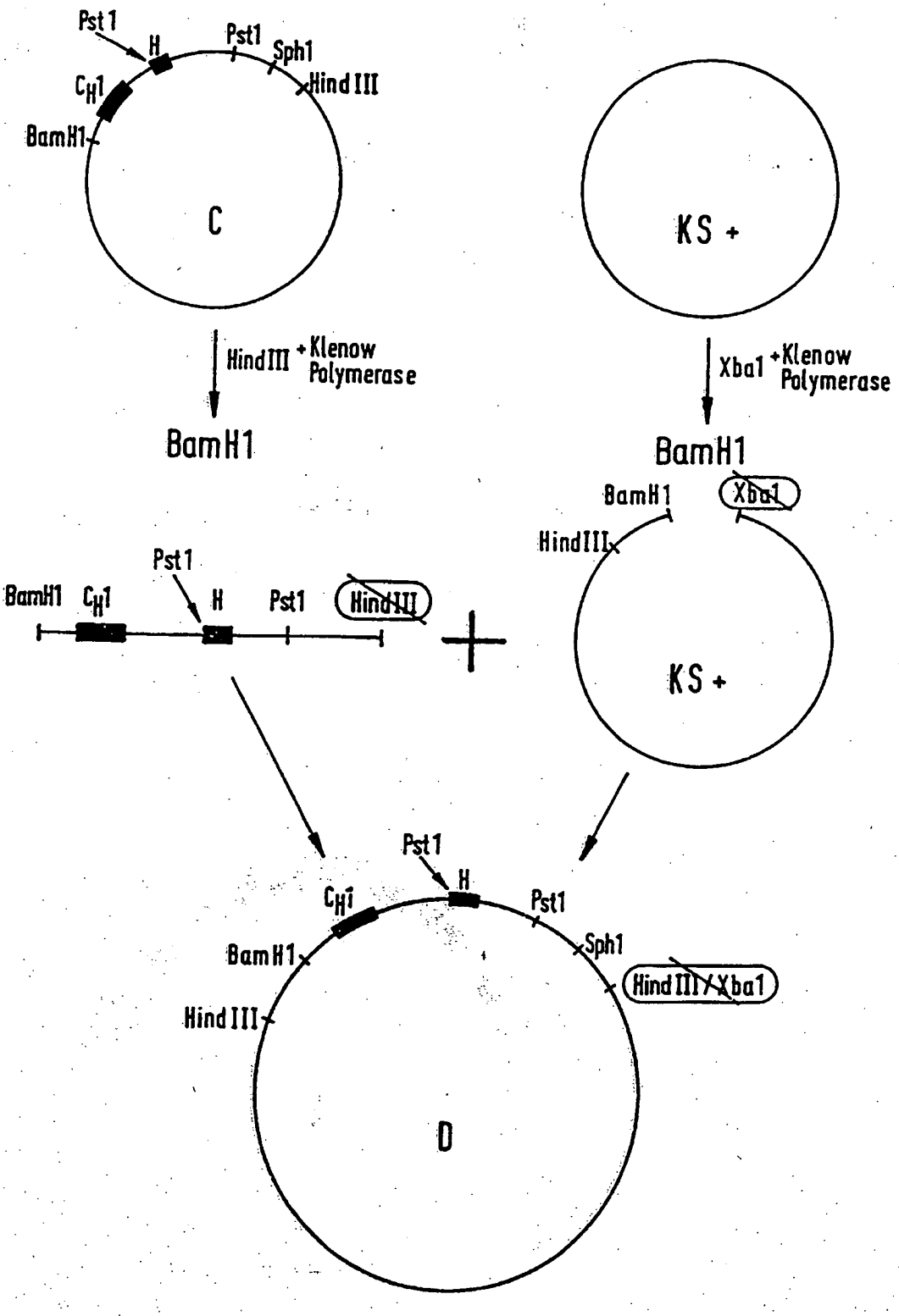
H
Pst1
Bam H1
Eco R1
C_H1
HindIII
Jg G 3 C
$F(ab')_2$ 1H
pUC 19
HindIII fill in
Pst1 partiell
Hind II
Pst1
HindIII
C_H1
Pst1
H
Pst1
pUC 18
T_4 Ligase
Pst1
H
Pst1
C_H1
Sph1
Hind III
HindII/HindIII
Bam H1
C

Fig.4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H
Pst1
Sph1
Hind III
C_H1
BamH1
C
KS +
Hind III + Klenow Polymerase
Xba1 + Klenow Polymerase
BamH1
BamH1
Xba1
Hind III
Pst1
BamH1
C_H1
H
Pst1
Hind III
KS +
Pst1
H
C_H1
Pst1
Sph1
BamH1
Hind III / Xba1
Hind III
D

Fig.5

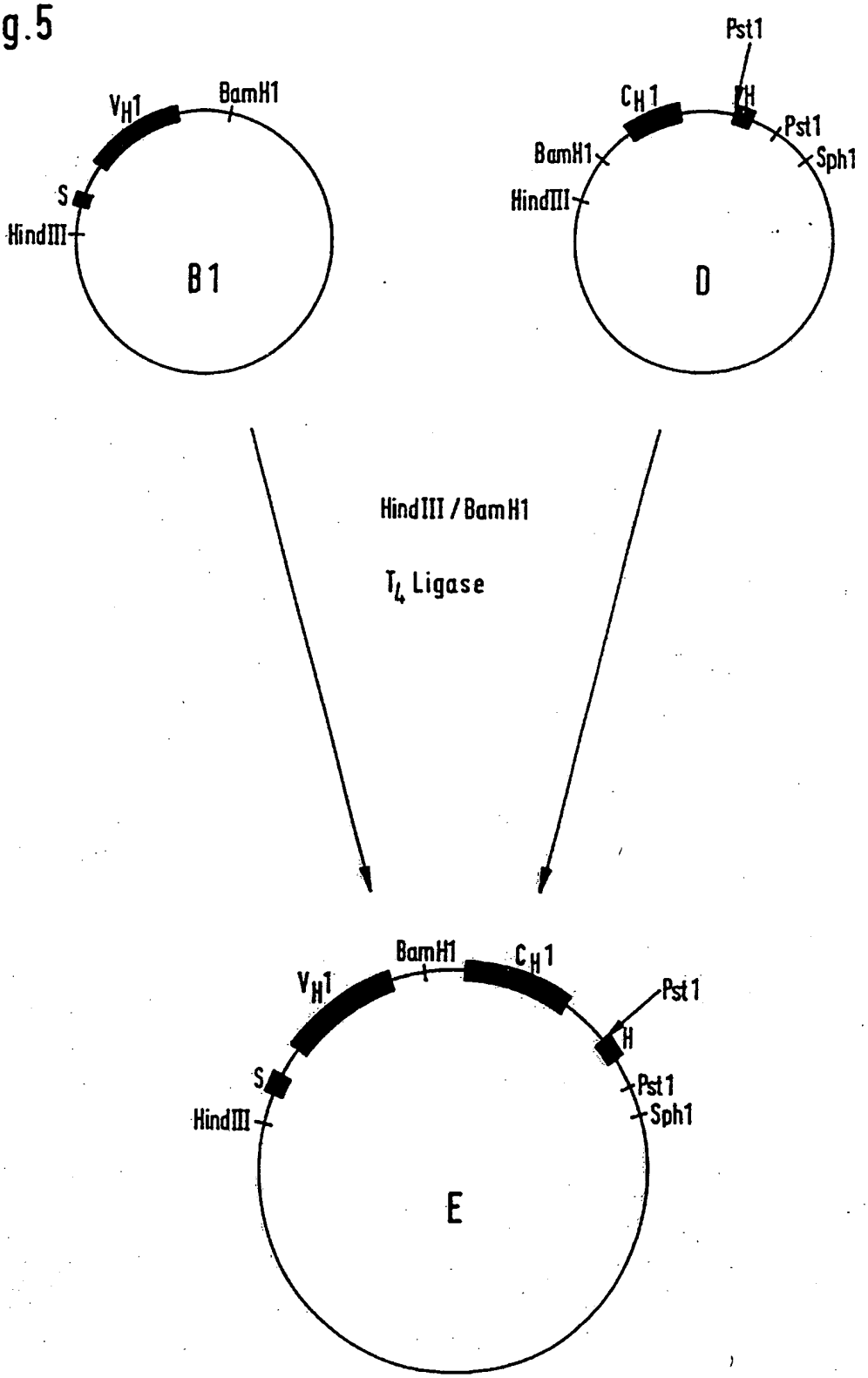

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Fig.6

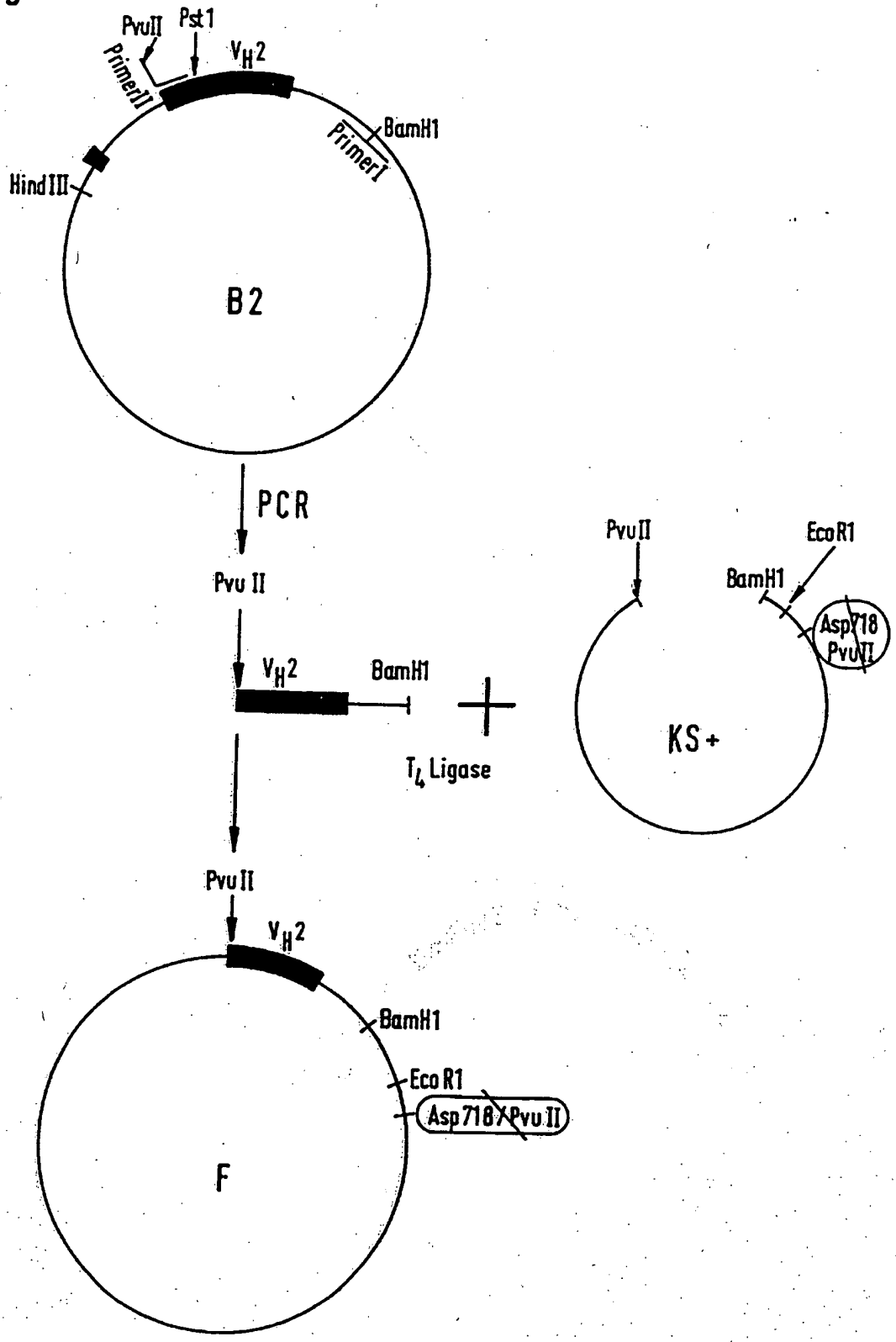

PvuII
Pst1
Primer II
V_H2
BamH1
Primer I
HindIII
B2
PCR
Pvu II
V_H2
BamH1
T_4 Ligase
PvuII
EcoR1
BamH1
Asp718
PvuII
KS+
PvuII
V_H2
BamH1
EcoR1
Asp718 PvuII
F

Fig.7

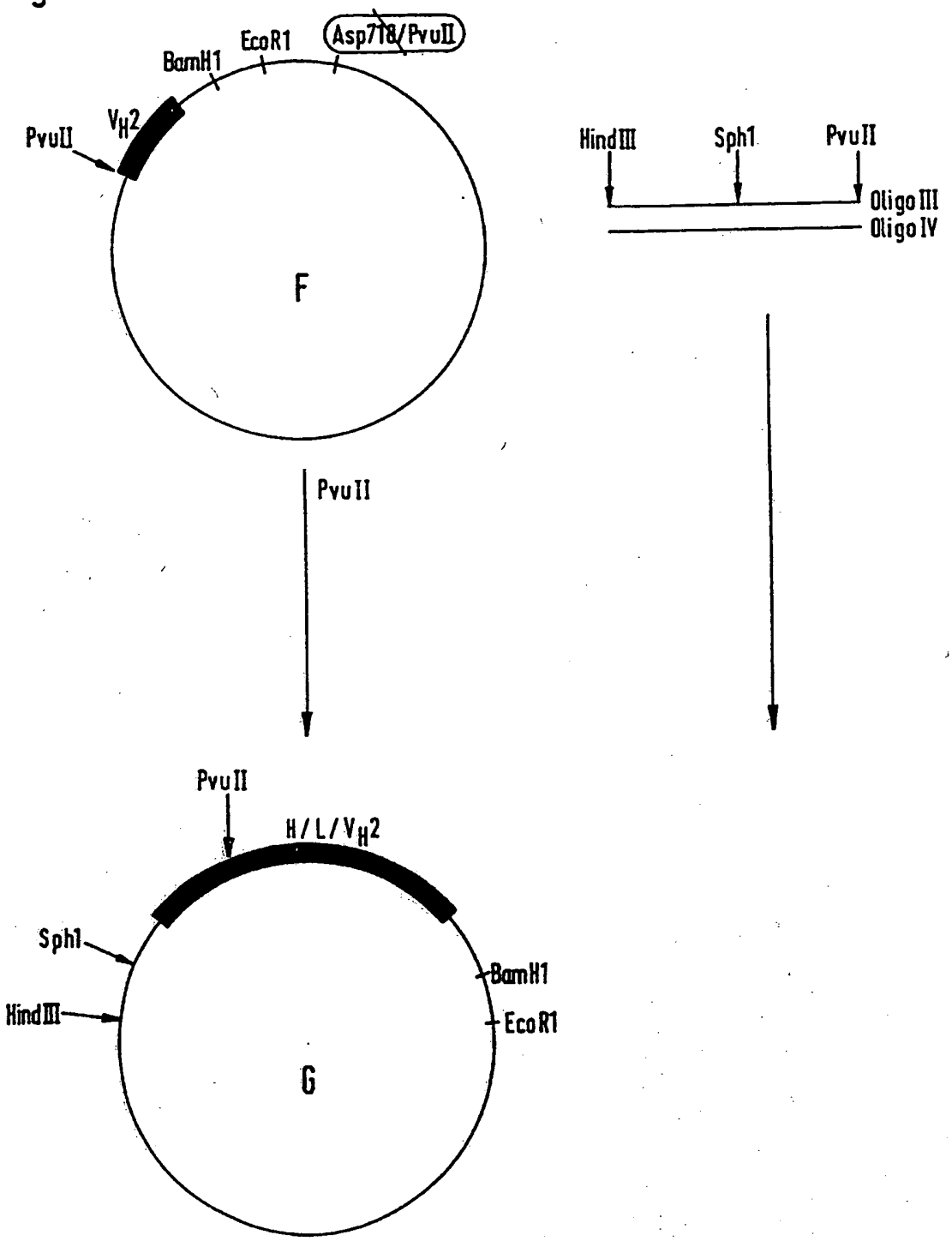
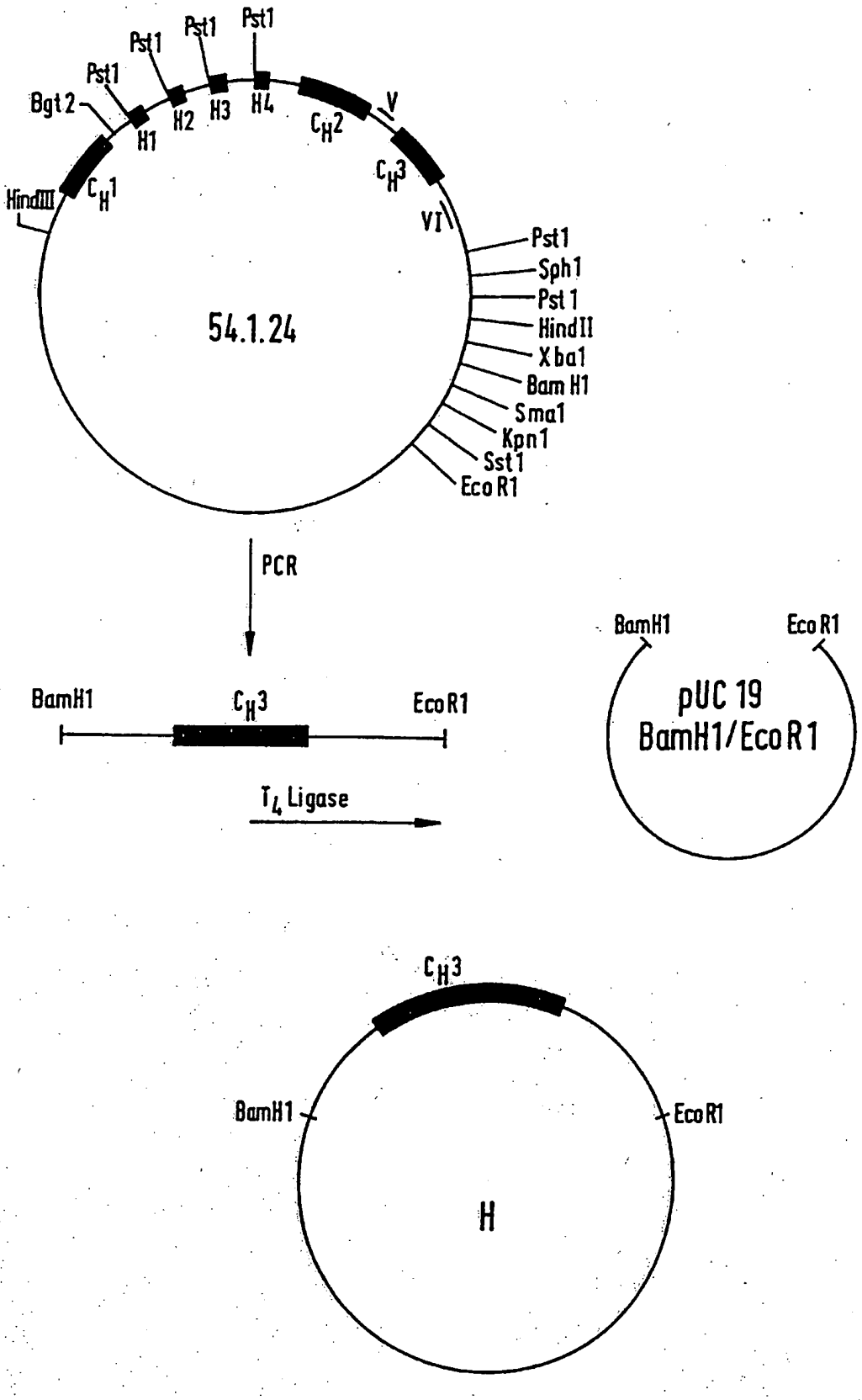


Fig.8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Pst1
Pst1
Pst1
Pst1
Bgt2
H1
H2
H3
H4
C_H2
V
C_H3
VI
C_H1
HindIII
54.1.24
Pst1
Sph1
Pst 1
HindII
Xba1
Bam H1
Sma1
Kpn1
Sst1
EcoR1
PCR
BamH1
C_H3
EcoR1
T_4 Ligase
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C_H3
BamH1
EcoR1
H

Fig.9

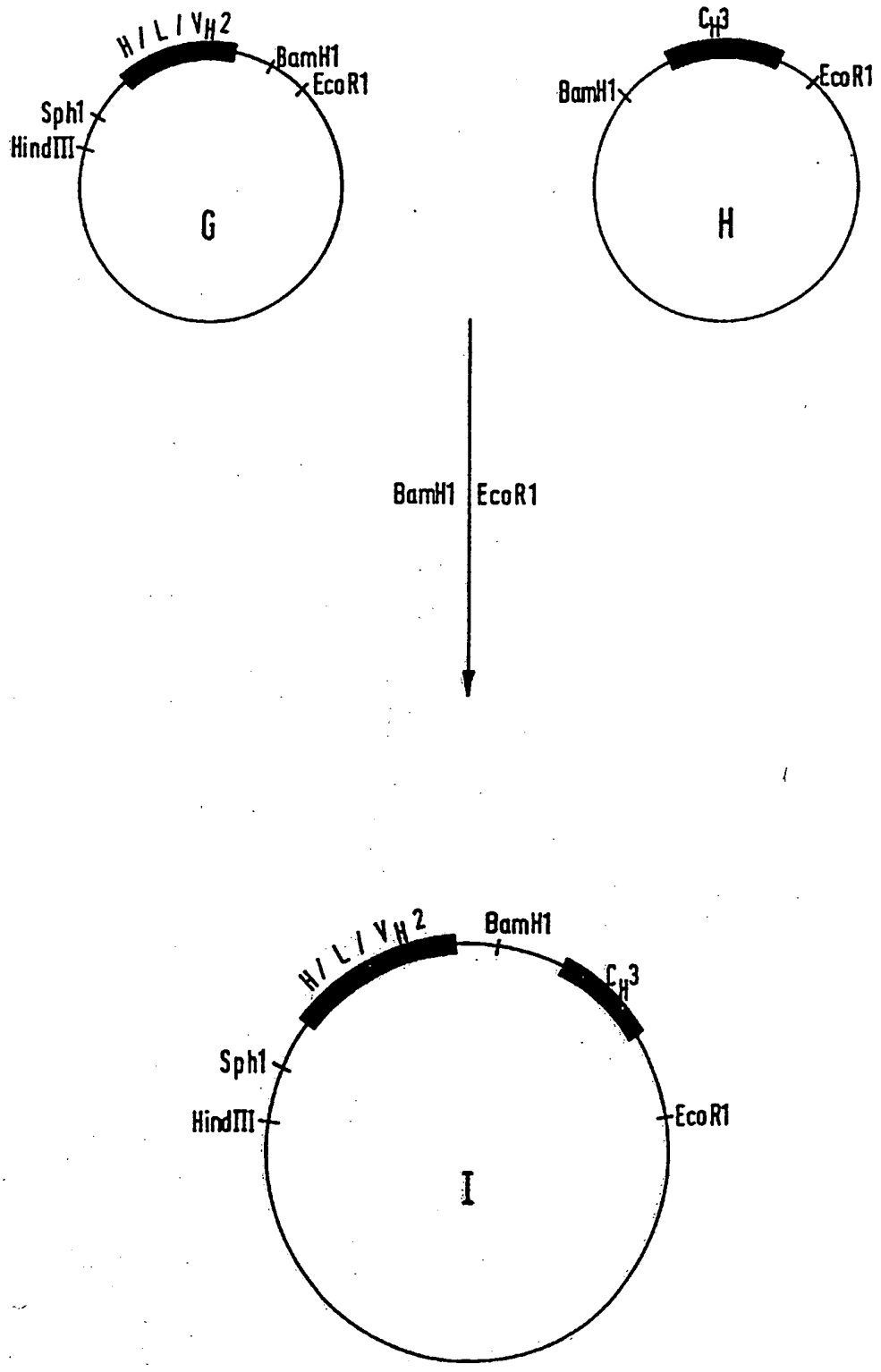
H/L/VH2
BamH1
EcoR1
Sph1
HindIII
G
CH3
BamH1
EcoR1
H
BamH1 EcoR1
H/L/VH2
BamH1
CH3
Sph1
HindIII
EcoR1
I

Fig.10

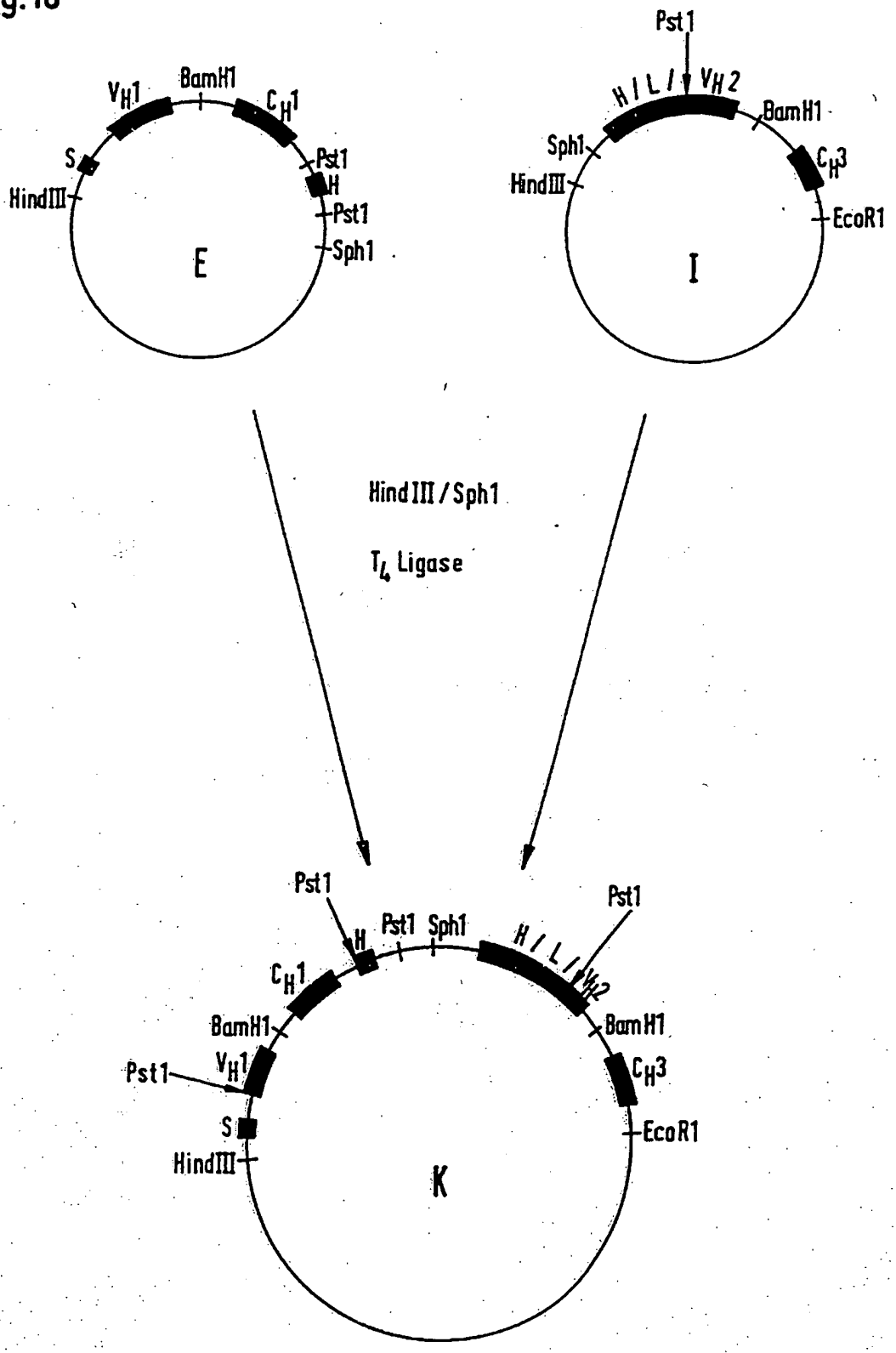
VH1
BamH1
CH1
S
Pst1
H
HindIII
Pst1
Sph1
E
Pst1
H I L I VH2
BamH1
Sph1
CH3
HindIII
EcoR1
I
HindIII / Sph1
T4 Ligase
Pst1
H
Pst1
Sph1
H I L I VH2
Pst1
CH1
BamH1
BamH1
VH1
Pst1
CH3
S
EcoR1
HindIII
K

Fig.11

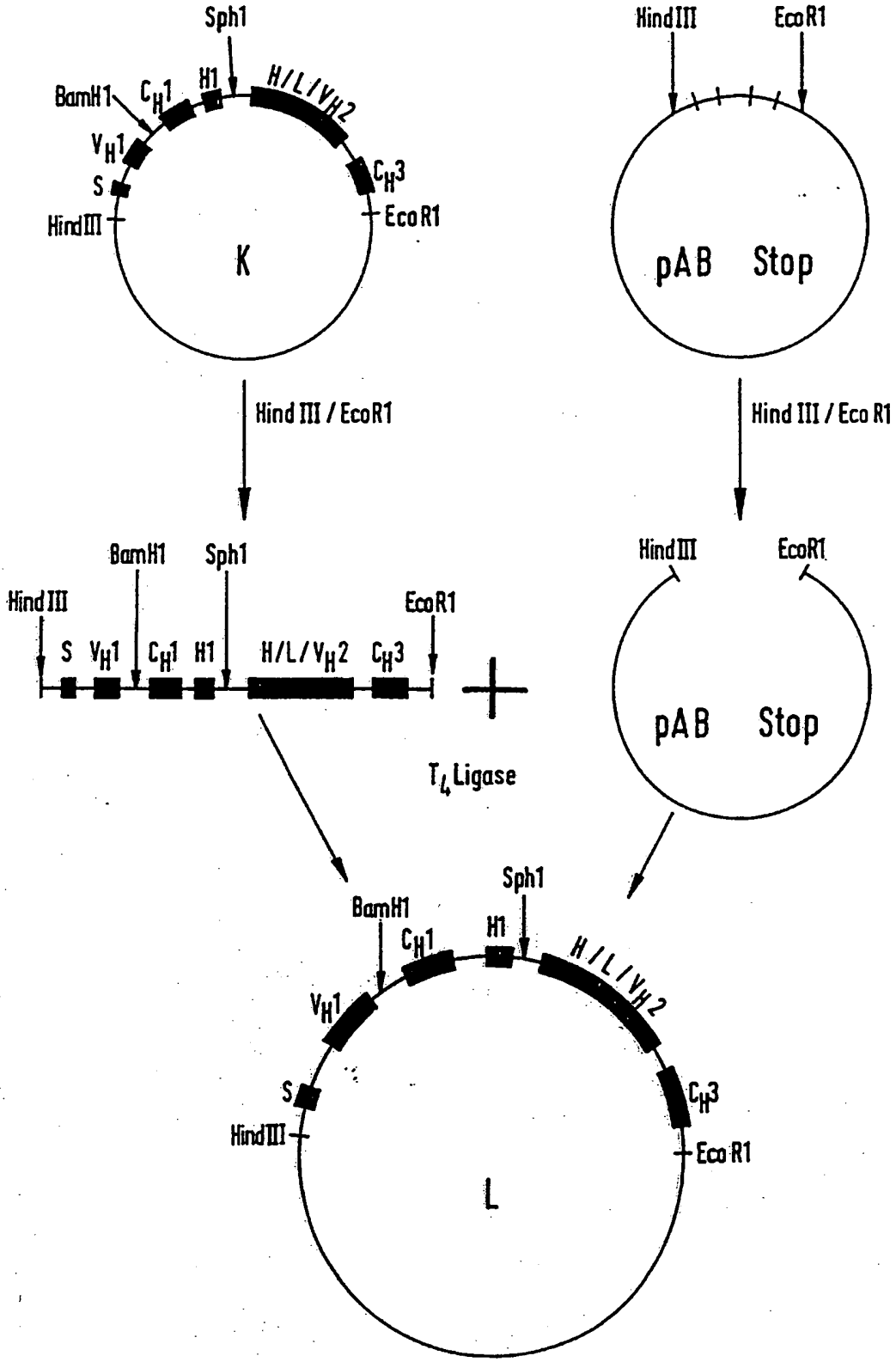
Sph1
BamH1
C_H1
H1
H/L/V_H2
V_H1
S
C_H3
HindIII
EcoR1
K
HindIII
EcoR1
pAB Stop
Hind III / EcoR1
Hind III / Eco R1
BamH1
Sph1
HindIII
EcoR1
S V_H1 C_H1 H1 H/L/V_H2 C_H3
HindIII
EcoR1
pAB Stop
T_4 Ligase
Sph1
BamH1
C_H1
H1
H/L/V_H2
V_H1
S
C_H3
HindIII
Eco R1
L

Fig.12

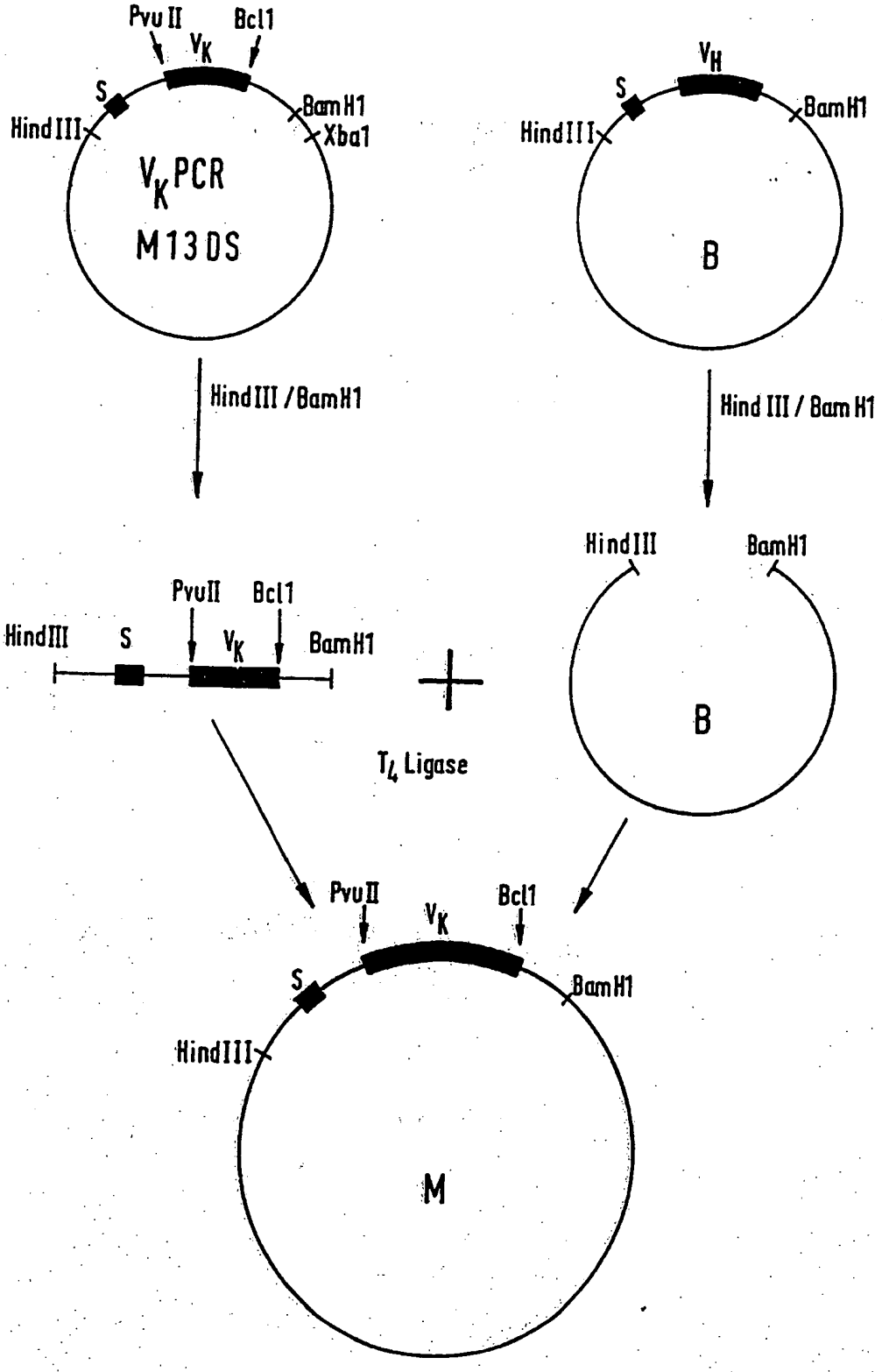

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Fig. 13

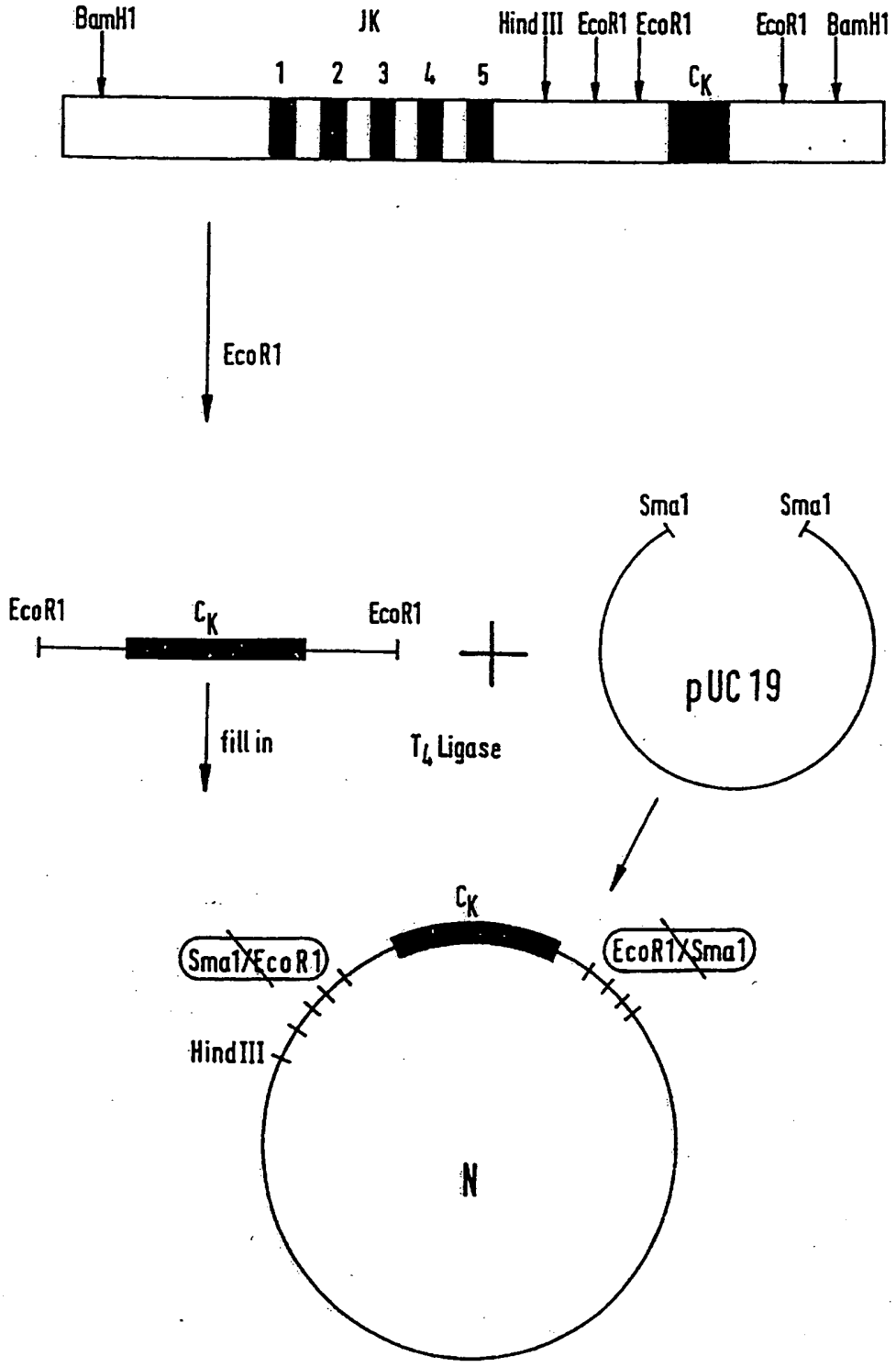

Fig. 14

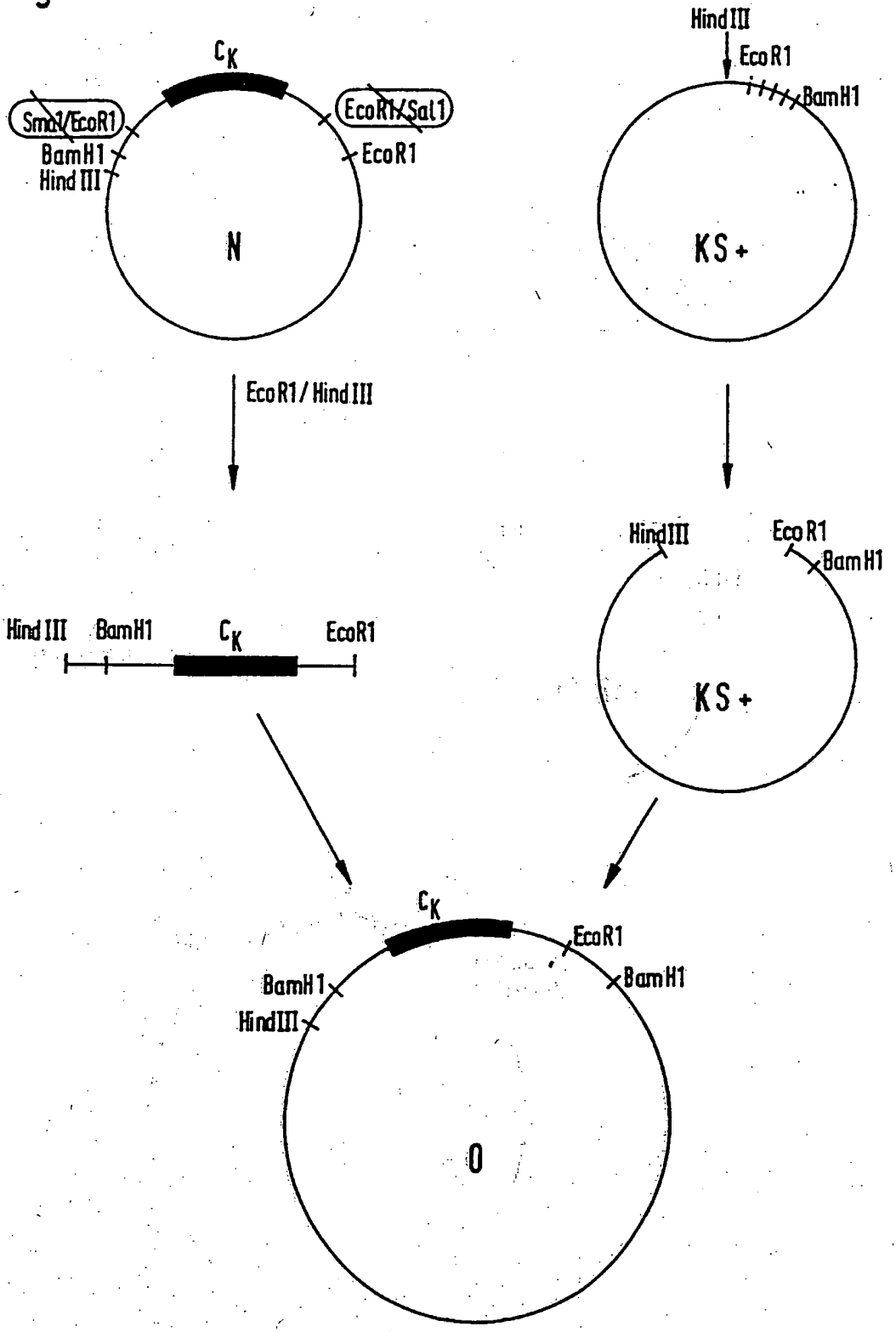
C_K
Sma1/EcoR1
BamH1
Hind III
EcoR1/Sal1
EcoR1
N
Hind III
EcoR1
BamH1
KS+
EcoR1/Hind III
Hind III
BamH1
C_K
EcoR1
Hind III
EcoR1
BamH1
KS+
C_K
EcoR1
BamH1
BamH1
Hind III
O

Fig.15

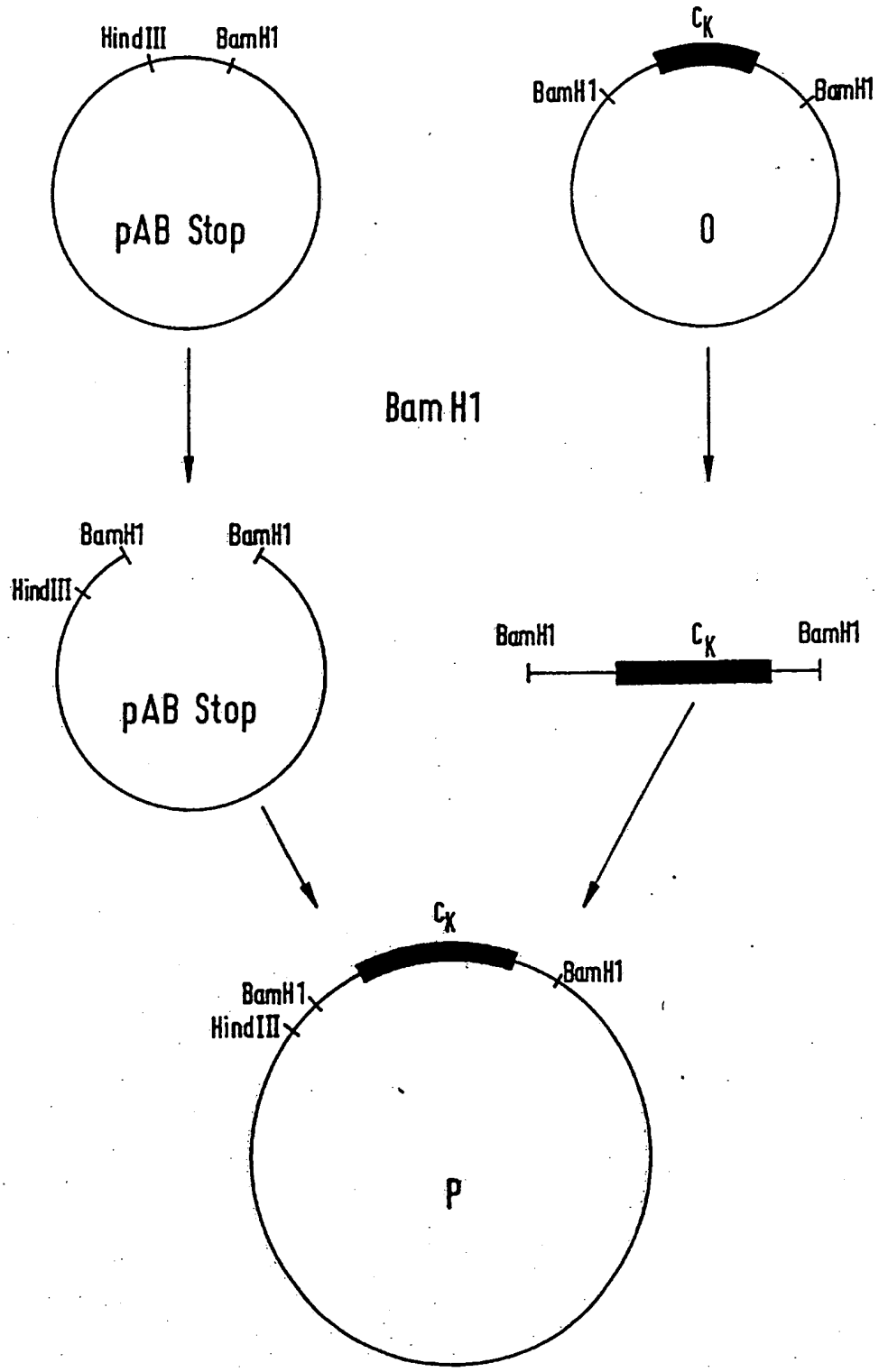
HindIII
BamH1
pAB Stop
C_K
BamH1
BamH1
O
Bam H1
BamH1
BamH1
HindIII
pAB Stop
BamH1
C_K
BamH1
C_K
BamH1
BamH1
HindIII
P

Fig.16

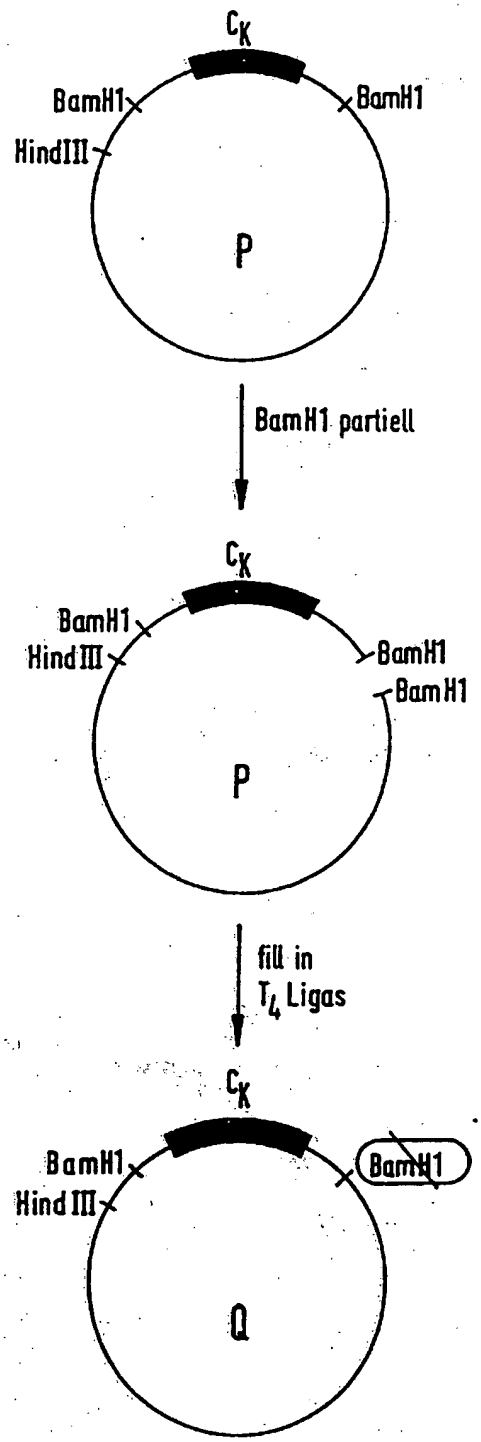
C_K
BamH1
BamH1
HindIII
P
BamH1 partiell
C_K
BamH1
HindIII
BamH1
BamH1
P
fill in
T_4 Ligas
C_K
BamH1
BamH1
HindIII
Q

Fig.17

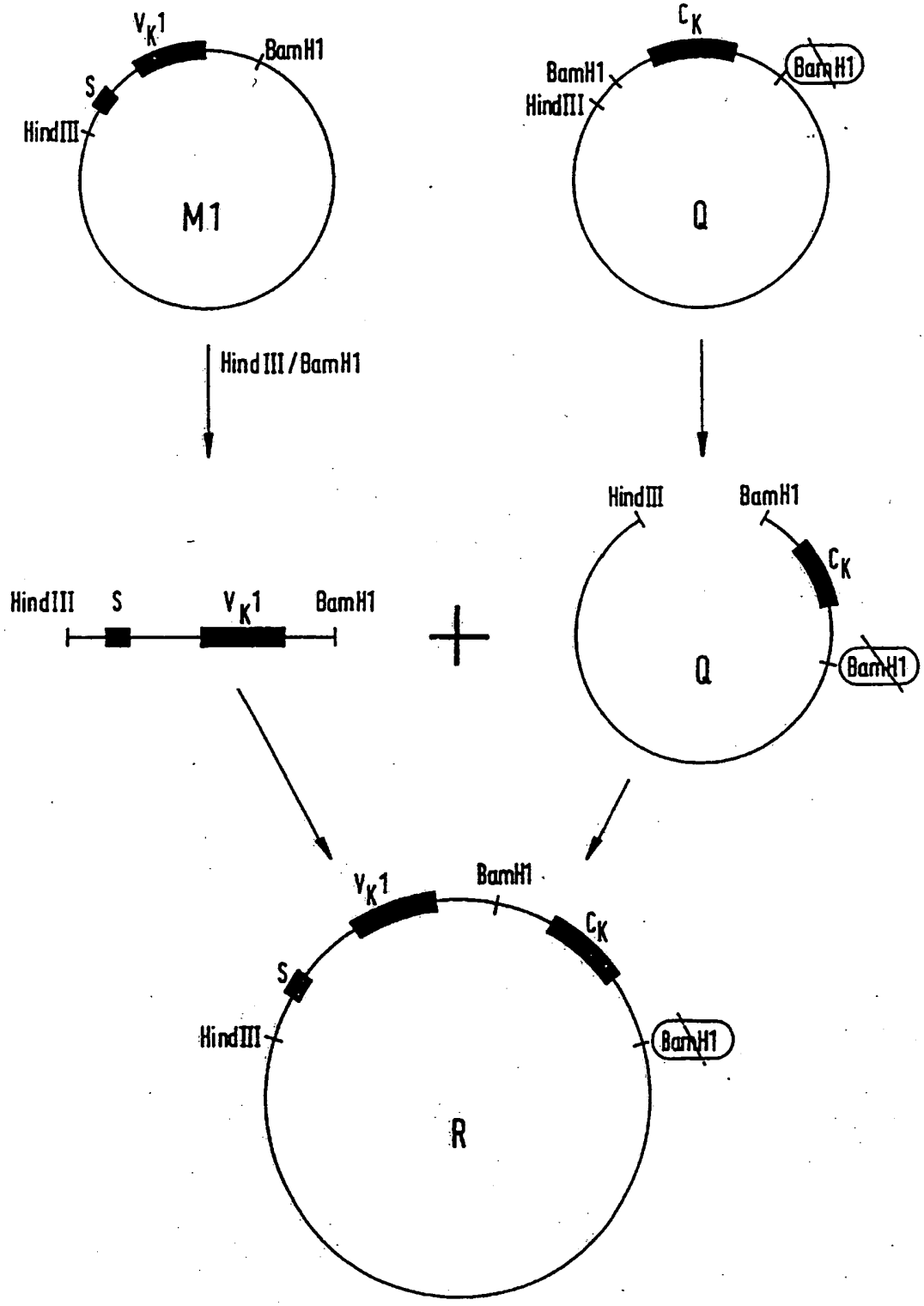
V_K1
BamH1
S
HindIII
M1
C_K
BamH1
HindIII
BamH1
Q
HindIII / BamH1
HindIII
S
V_K1
BamH1
HindIII
BamH1
C_K
Q
BamH1
V_K1
BamH1
C_K
S
HindIII
BamH1
R

Fig.18

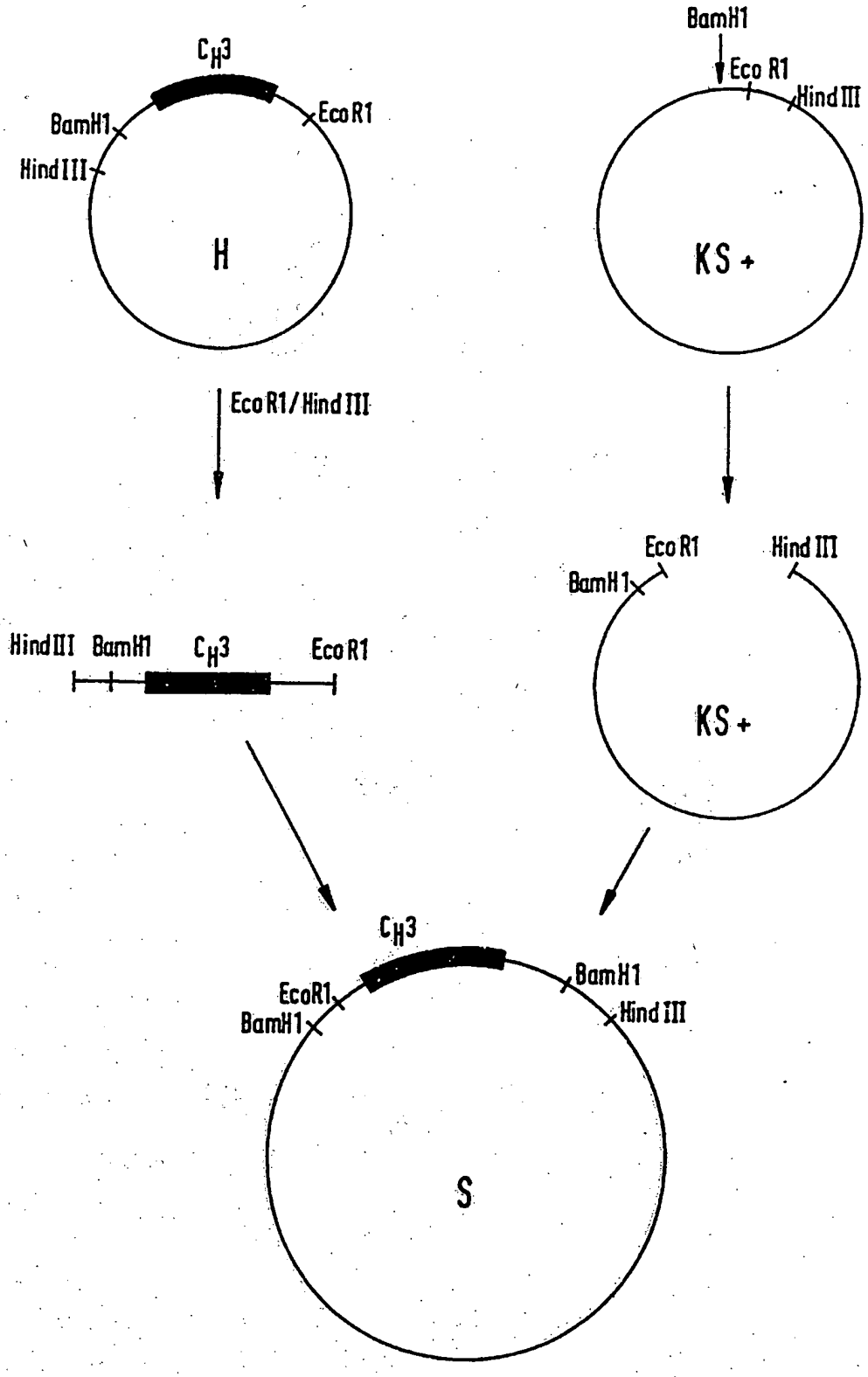

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Fig.19

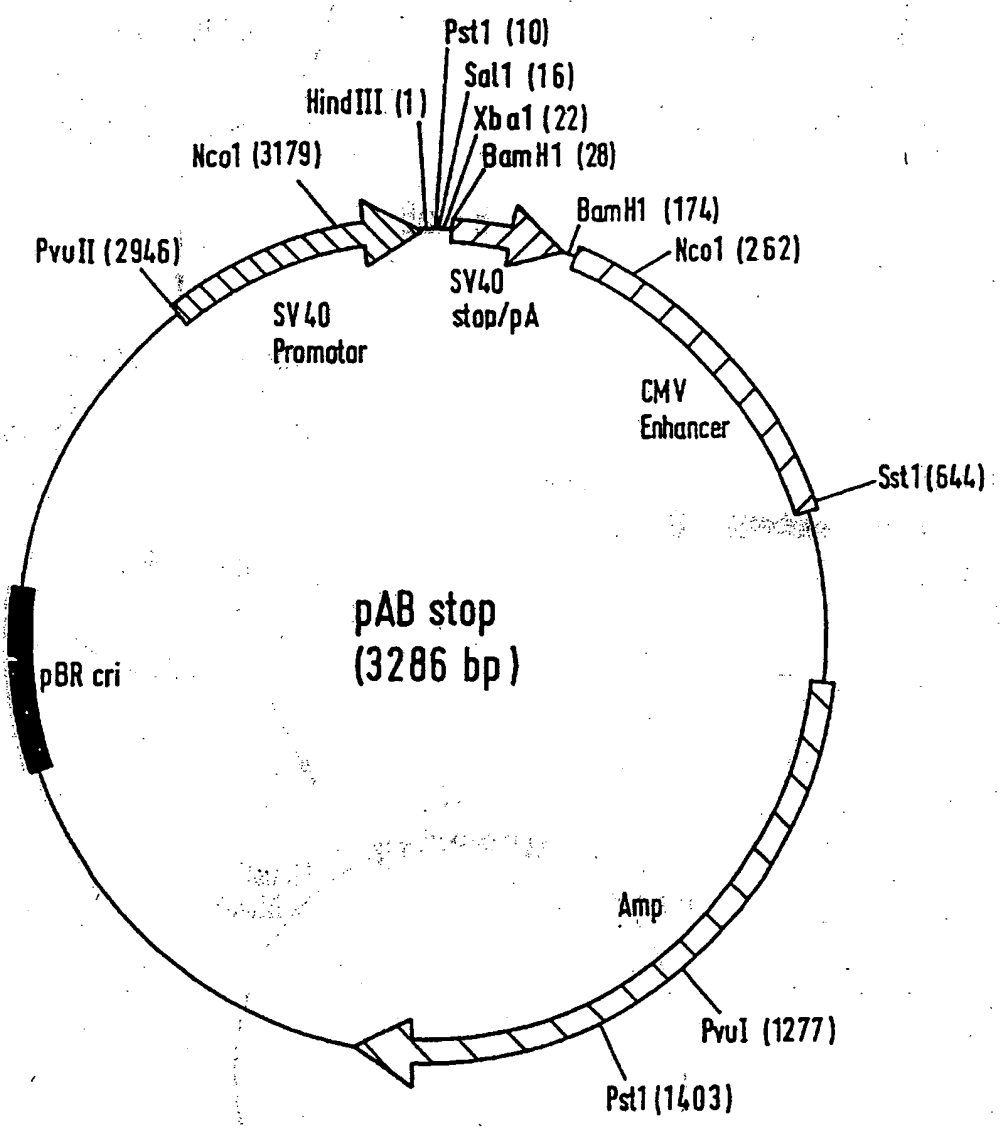

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Fig.20

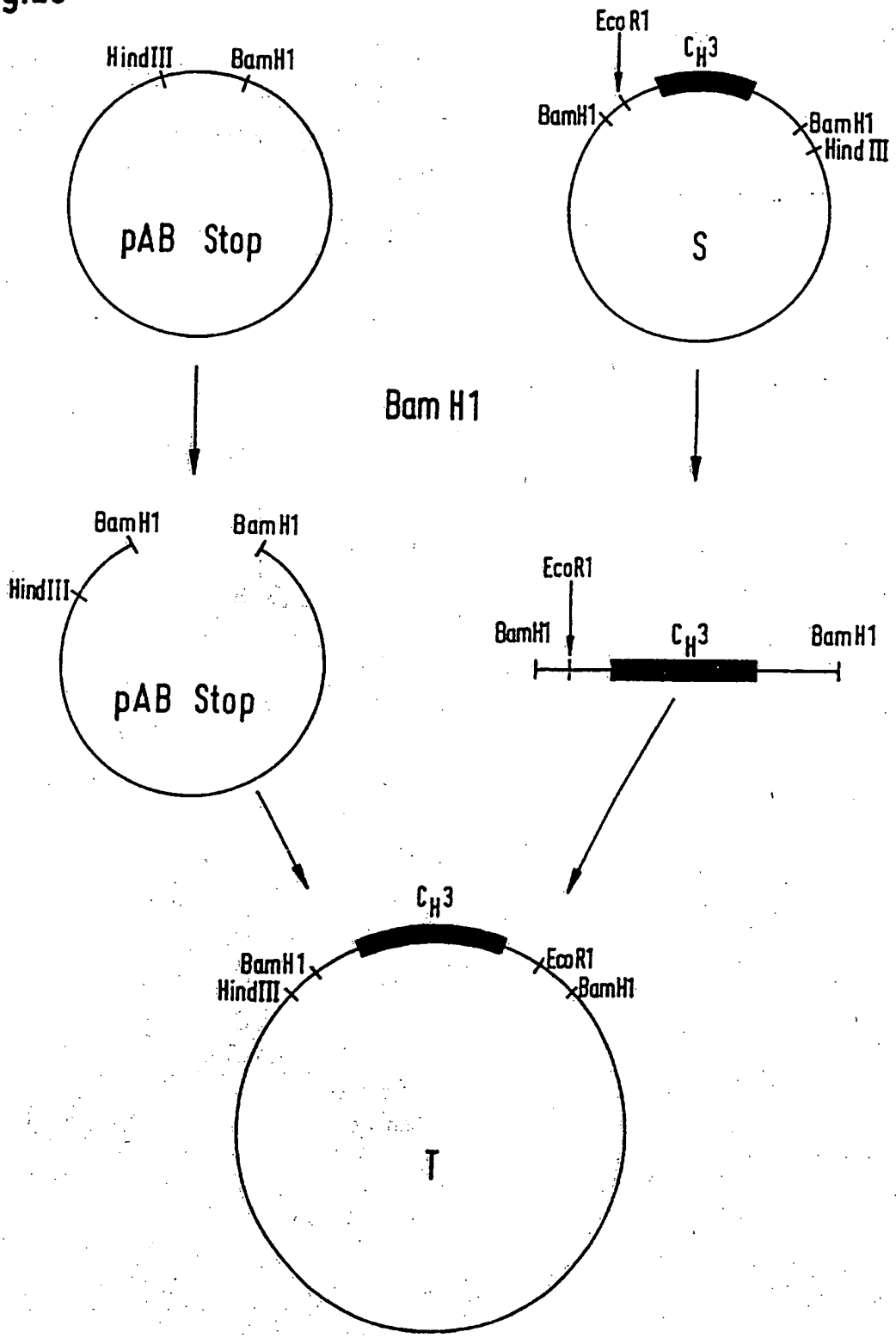
HindIII
BamH1
pAB Stop
EcoR1
C_H3
BamH1
BamH1
Hind III
S
Bam H1
BamH1
BamH1
HindIII
pAB Stop
EcoR1
BamH1
C_H3
BamH1
C_H3
BamH1
HindIII
EcoR1
BamH1
T

Fig. 21

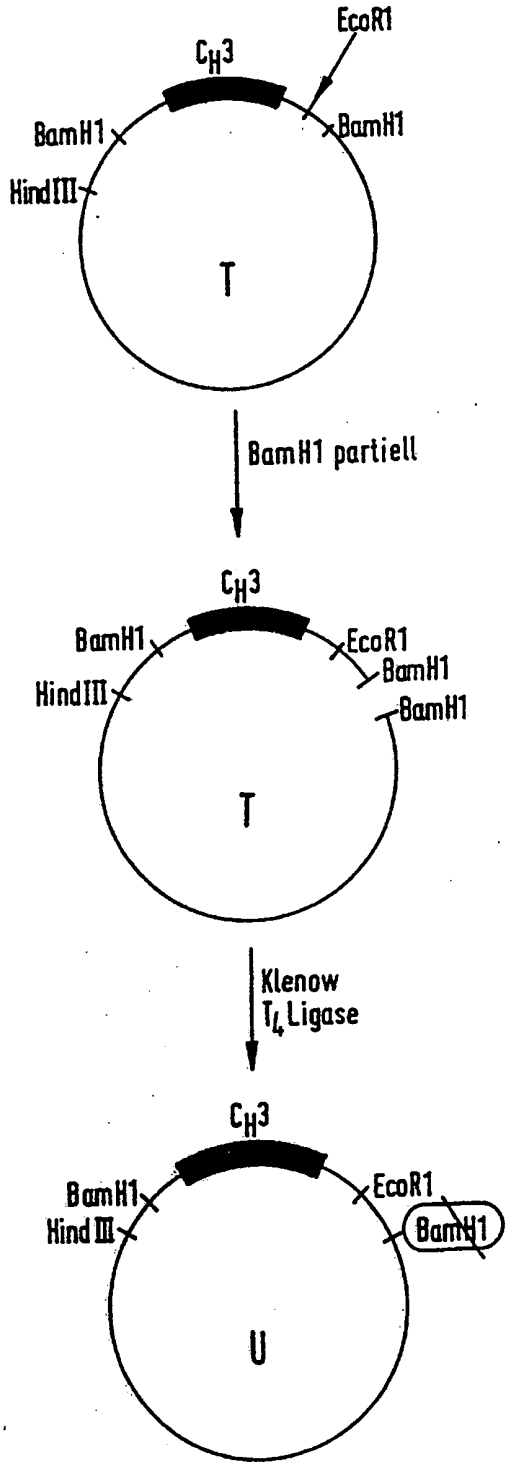
EcoR1
C_H3
BamH1
BamH1
HindIII
T
BamH1 partiell
C_H3
BamH1
EcoR1
BamH1
HindIII
BamH1
T
Klenow
T_4 Ligase
C_H3
BamH1
EcoR1
Hind III
BamH1
U

Fig.22

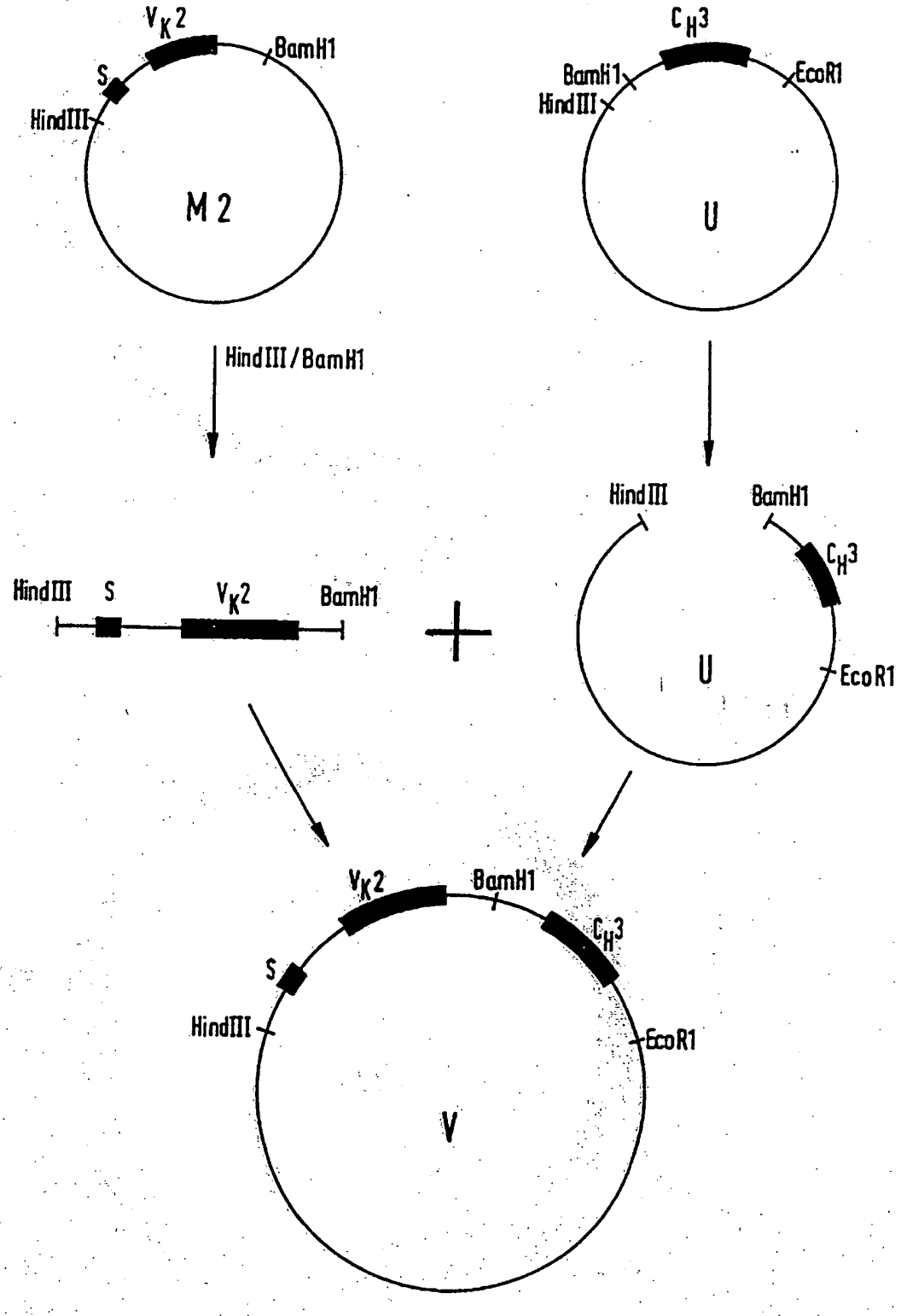

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Fig.23

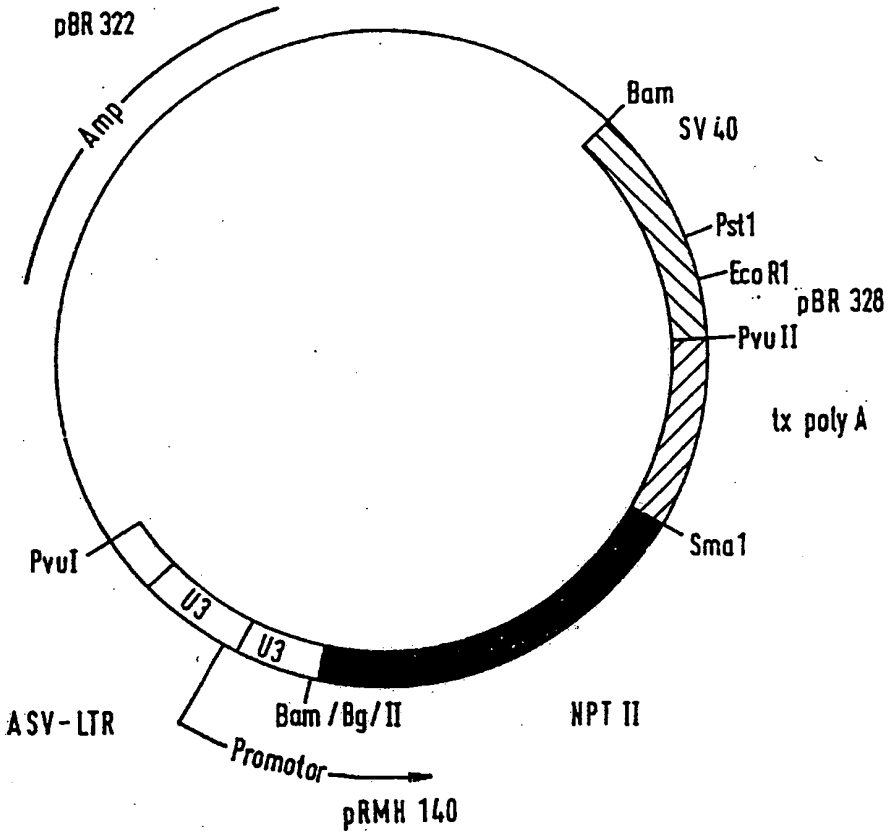
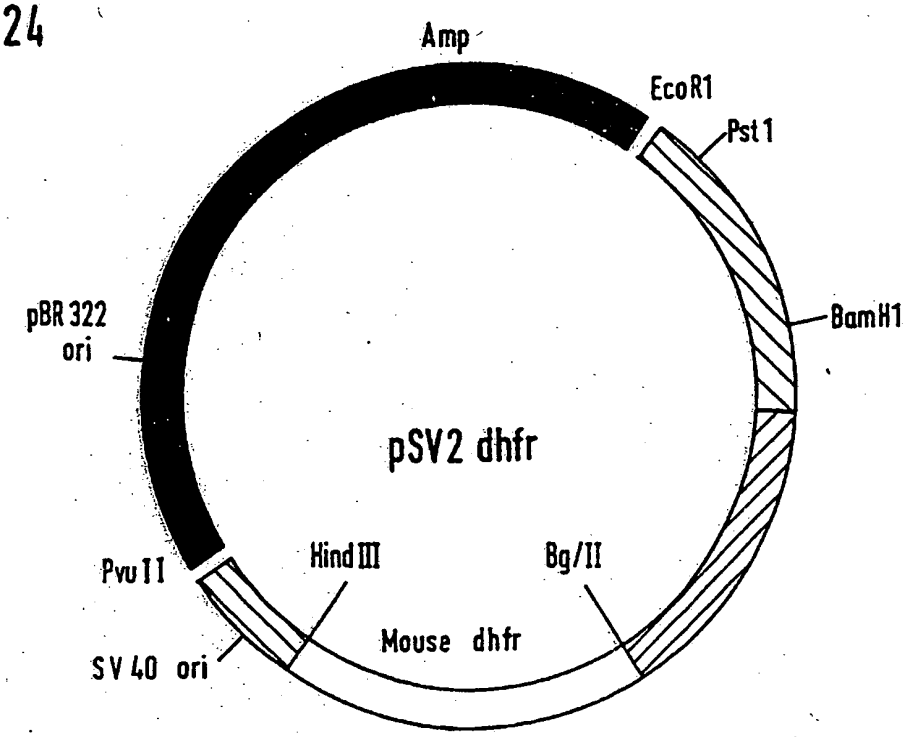

pBR 322
Amp
Bam
SV 40
Pst1
Eco R1
pBR 328
Pvu II
tx poly A
Sma1
PvuI
U3
U3
ASV-LTR
Bam / Bg / II
NPT II
Promotor
pRMH 140

Fig.24


Amp
EcoR1
Pst1
BamH1
pBR 322
ori
pSV2 dhfr
Pvu II
Hind III
Bg/II
Mouse dhfr
SV 40 ori